IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Latta, Paul P.
Appl. No.	:	10/823,263
Filed	:	April 13, 2004
For	:	A METHOD OF TREATMENT OF DIABETES THROUGH INDUCTION OF IMMUNOLOGICAL TOLERANCE
Examiner	:	Belyavski, Michail A.
Group Art Unit	:	1644

DECLARATION OF DAVID SCHARP, M.D.

UNDER 37 C.F.R §1.132

Commissioner for Patents

P.O. Box 1450

Alexandria, VA 22313-1450

Dear Sir:

1. I, David Scharp, M.D., am Executive Vice President and Chief Medical Officer of Novocell, Inc. At Novocell I am actively engaged in research related to development of treatments for diabetes using encapsulated insulin-producing cells.

2. I have extensive experience in the field of the claimed invention as indicated in the attached Curriculum Vitae provided herewith as Exhibit A.

3. Working with the sole inventor of the present application, Paul P. Latta, and others, I carried out experiments to evaluate the efficacy of treating diabetes through induction of immunological tolerance.

4. The currently available approach to treating diabetes, which does not require repeated administration of insulin, is transplantation of insulin-producing cells from a donor to the diabetic patient. However, the problem with this approach is that such transplants do not survive the attack of the host immune system unless the patients are under continuous, life-long immunosuppression.

5. The approach of the present invention is totally different in that we are implanting a very small fraction of a therapeutic dose of encapsulated islet allografts prior to implantation of the fully therapeutic dose in order to tolerize the immune system to the foreign antigens shed by these encapsulated allografts made impervious to the assault by the host immune system. The fraction of encapsulated islets in the tolerizing dose is so small it would have no effect on controlling blood glucose by itself. However, the continuous release of donor antigens from this small fraction of encapsulated islets not destroyed by the host, alters the immune response in a way that the host no longer considers the implanted islets to be foreign. The tolerization therefore, protects the later implanted non-encapsulated islet allografts from being rejected by the host, allowing them to take permanent hold in the host body and produce the fully therapeutic amount of insulin as needed to treat diabetes.

6. In our original experiments, described in the Specification of the above-identified application, we induced diabetes in mice by intravenous injection of streptozotocin. Induction of diabetes by streptozotocin injection is a well-known procedure which destroys pancreatic insulin-producing cells. We first implanted into these mice a small, sub-therapeutic dose of encapsulated insulin-producing cells. Two to three weeks later the same mice received an additional, large (therapeutic) unencapsulated implant (2,000-3,000 cell aggregates, 1000 cells per aggregate) of insulin-producing cells. We showed that the encapsulated insulin-producing cells given as a small mass of 100 cell aggregates (1,000 cells per aggregate) permits this second unencapsulated implant to survive as shown by normalized blood glucose levels in the treated mice.

7. We then turned to another animal model of diabetes, non-obese diabetic (NOD) mice, and proved that the method works in this model as well.

8. There are two available animal models for studying Type I diabetes, the BB rat and the NOD mouse. The BB rat, while developing diabetes, has a multitude of immunologic disorders that makes it more of a model for immune deficiency than diabetes. The BB rat is no longer considered an acceptable model for studying human autoimmune diabetes.

9. The NOD mouse is therefore, the only animal model for autoimmune, Type I diabetes that it is predictive of human disease. The lymphocytes of the NOD mouse spontaneously begin attacking its insulin-producing pancreatic beta cells soon after birth. Looking at the histology of the pancreas of these mice during the autoimmune process, one finds early that most islets are infiltrated with immune cells that are destroying islets. As the process continues towards the diabetic phase, almost all of the beta cells are destroyed leaving smaller than normal islets with residual inflammation that continues to destroy the new islets that are stimulated to develop due to the failing islet mass. This ongoing destruction of the insulin-producing pancreatic beta cells continues and progresses for 15 to 32 weeks until a sufficient number of beta cells are destroyed to cause the clinical onset of Type I diabetes in NOD mice. Examining living NOD mice prior to development of clinical diabetes, including monitoring their blood glucose levels, one would have no clue that this autoimmune process is actively destroying their pancreatic beta cells. Yet, if one examines their blood for anti-islet protein antibodies, one can clearly identify those animals that will eventually lose blood glucose control and develop clinical diabetes. This situation is identical for human Type I diabetes in that patients at high risk for developing Type I diabetes are tested for the presence of specific auto-antibodies. The number and titers of these specific antibodies can predict with >90% certainty which of these patients with ongoing autoimmune destruction of their beta cells will actually develop clinical Type I diabetes within 5 years.

10. We encapsulated islets from mouse strain C57Bl/6 by polyethylene glycol conformal coating as described in USP 5,529,914. This patent was incorporated by reference into the specification of the application captioned above. We then implanted these conformally-coated islets by intraperitoneal injection into NOD mice. The experiment had two variables under study

– time of implantation of the encapsulated islets (4, 8, and 12 weeks) and number of islets implanted (50, 100 and 150 islet equivalents, IEQ).

11. **Exhibit 1** shows the data for all animals grouped by time of implantation. Implantation at 4 weeks showed the best results with diabetes being prevented in 60% of the treated animals, as compared to the control animals with none having diabetes prevented.

12. **Exhibit 2** shows the data for all animals grouped by number of islets implanted. A dose of 50 IEQ produced the best results with diabetes being prevented in 60% of the treated animals. All the control animals developed diabetes.

13. **Exhibit 3** demonstrates that in the control recipients (NOD mice not implanted with encapsulated islet cells), the autoimmune destruction of the pancreatic islets is very complete with small shrunken islets remaining with continuing evidence of lymphocytes destroying any new islets that are formed.

14. **Exhibits 4 & 5** demonstrate that in those recipients that were prevented from developing diabetes after implanting the small quantity of encapsulated islets, very large islets (many times their normal size) are present, without evidence of host lymphocyte destruction. This means that the normal process in the mouse to replace lost islets has been successful to the point of preventing diabetes from destroying all of the newly formed islet cells.

15. Therefore, we have shown that: a) a small, sub-therapeutic tolerizing dose of encapsulated insulin-producing cells prevents the host immune system from attacking a later-implanted un-encapsulated therapeutic dose of insulin-producing cells; and b) a small, sub-therapeutic tolerizing dose of encapsulated insulin-producing cells prevents the host immune system from attacking the host insulin-producing cells in the pancreas.

16. Combined, these experiments prove that the claimed method of treating diabetes by tolerizing the host immune system prior to implanting the fully therapeutic dose of the insulin-producing cells works to permit the host to receive the fully therapeutic dose without rejection. This process works without the need of continuous, life-time immunosuppression of the host.

17. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these

statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or patent issuing therefrom.

Dated: May 4, 2005 By: _____

David Scharp

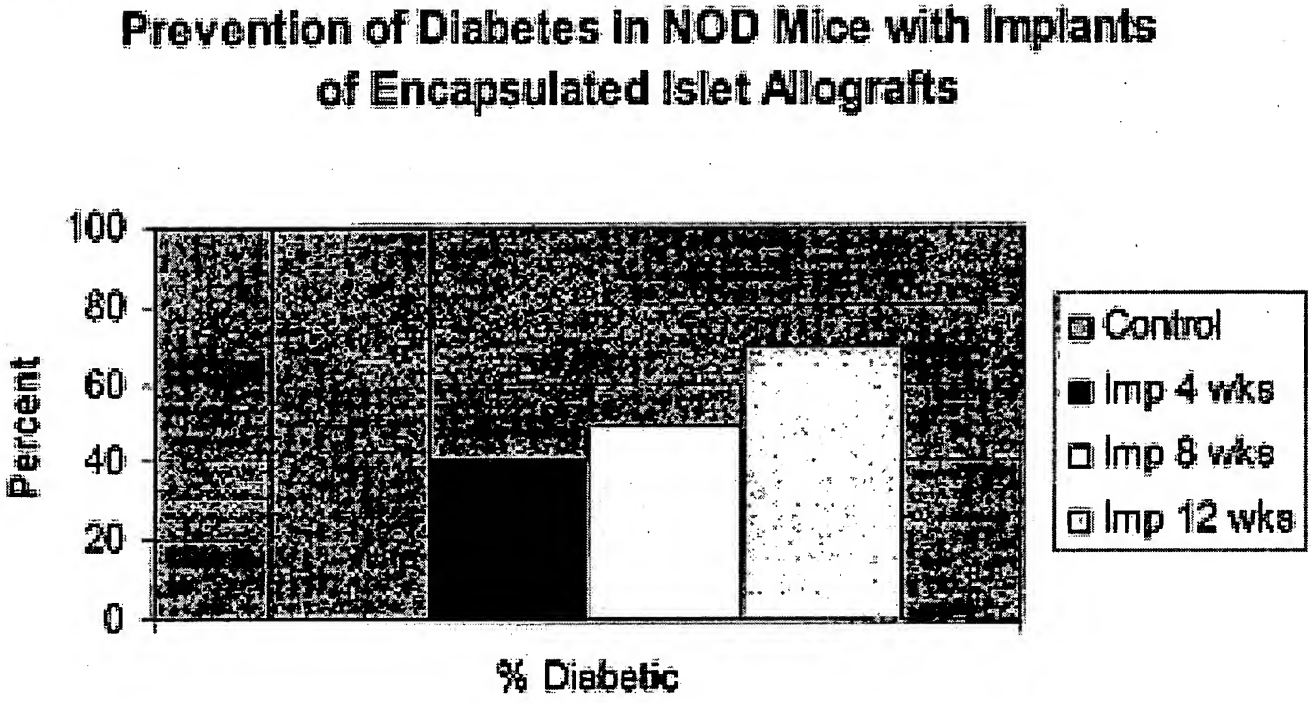

Prevention of Diabetes in NOD Mice with Implants of Encapsulated Islet Allografts
100
80
60
40
20
0
Percent
% Diabetic
Control
Imp 4 wks
Imp 8 wks
Imp 12 wks

Exhibit 1

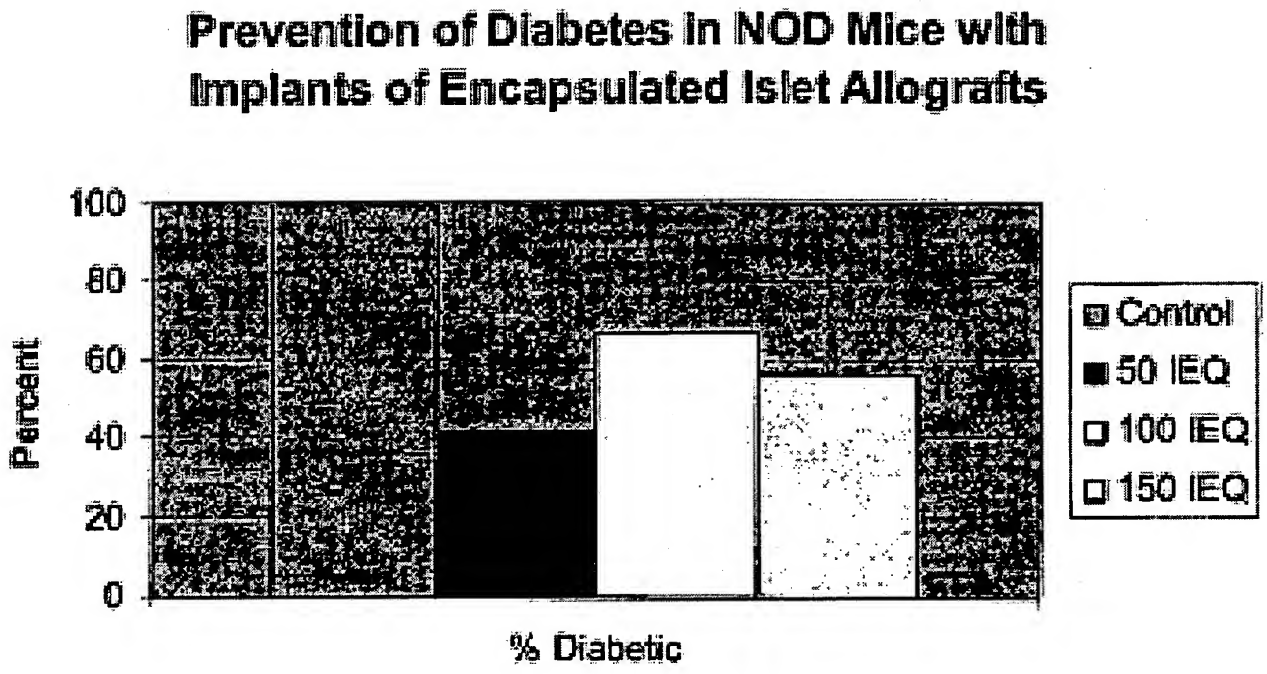
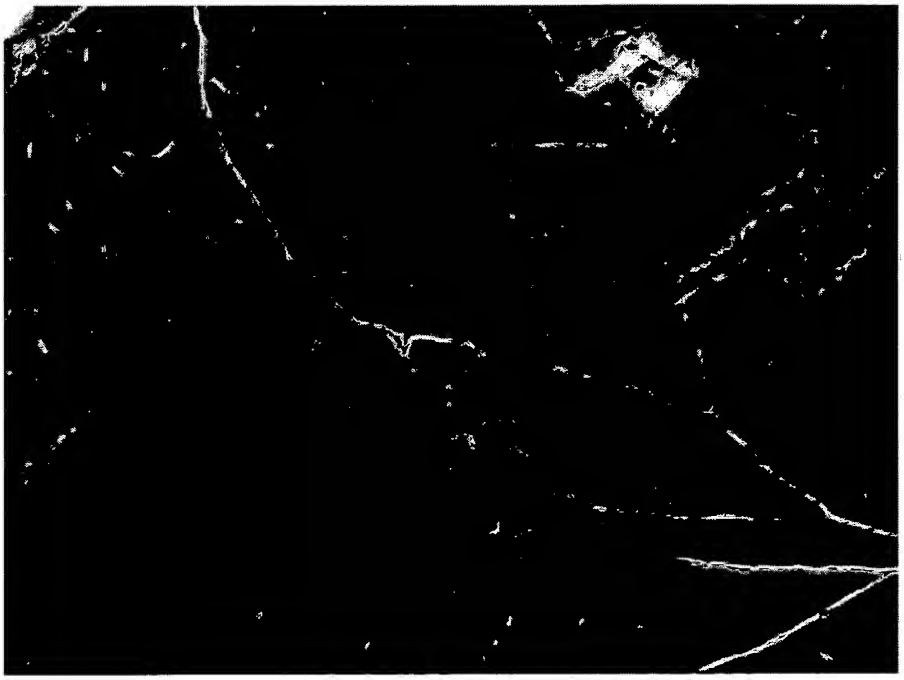

Prevention of Diabetes in NOD Mice with Implants of Encapsulated Islet Allografts
100
80
60
40
20
0
Percent
% Diabetic
Control
50 IEQ
100 IEQ
150 IEQ

Exhibit 2



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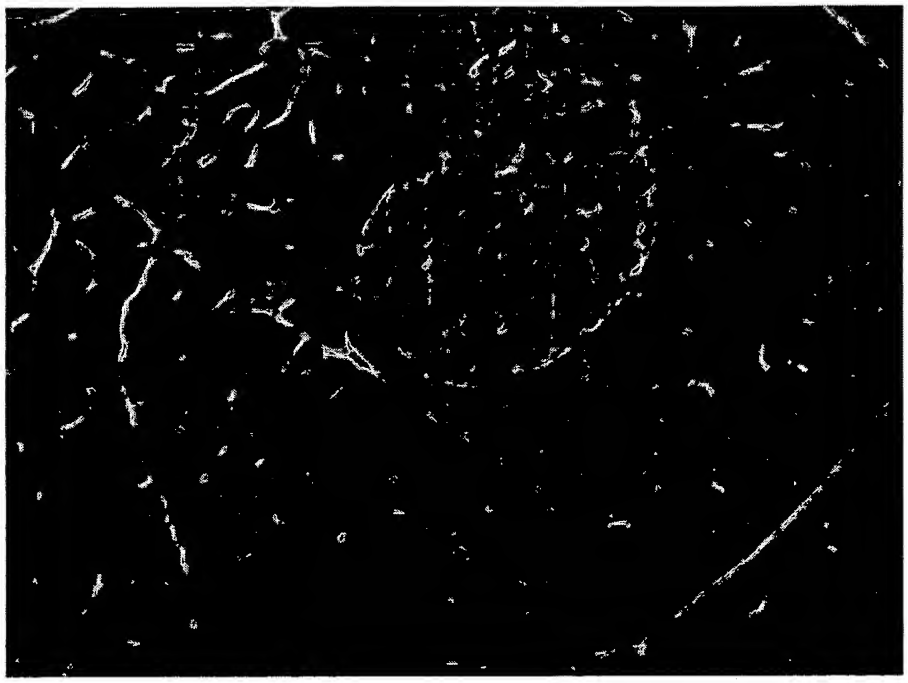

Exhibit 4

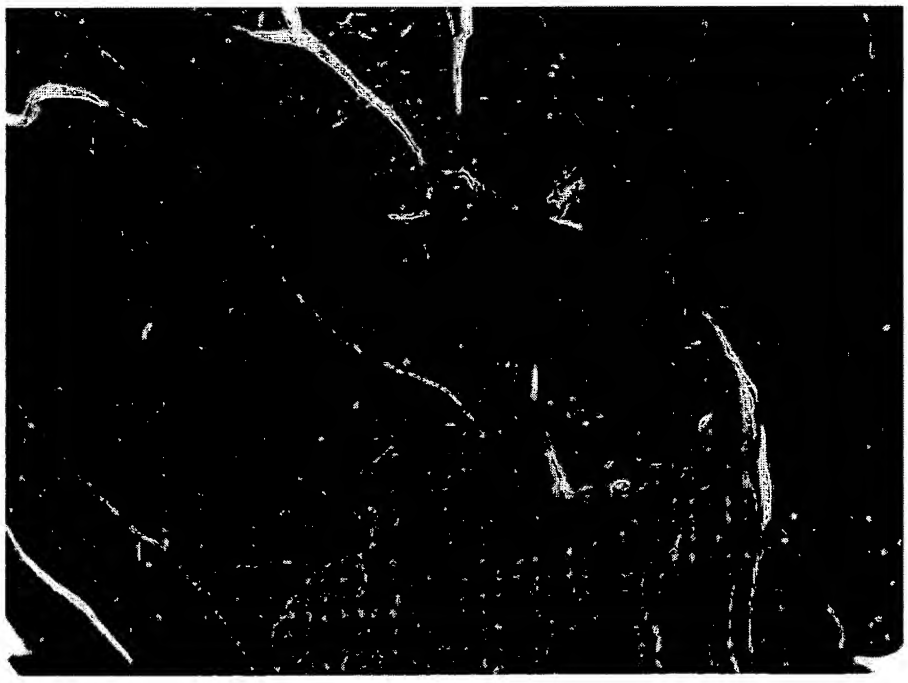

Exhibit 5

Exhibit A

CURRICULUM VITAE

David William Scharp, M.D.

Born: July 5, 1945
Washington, Illinois

Social Security Number: 327-36-7524

Marital Status: Wife: Jeanette

Children: Kevin Scharp
Daniel Scharp
Anna Scharp
David Bondurant
Melissa Bondurant

Pre-Medical Education: University of Missouri
Columbia, Missouri
1963-1966

Medical Education: Washington University School of Medicine
St. Louis, Missouri
1966-1970

Graduate Hospital Experience:

Intern in Surgery
7/1/70-6/30/71
Barnes Hospital/Washington University

Surgical Resident
7/1/71-6/30/72 & 7/1/74-6/30/76
Barnes Hospital/Washington University

Surgical Research Fellow
7/1/72-6/30/74
Washington University - Department of Surgery

Academic Positions:

Assistant Professor of Surgery
1976-1983
Washington University School of Medicine

Associate Professor of Surgery
1983-1991
Washington University School of Medicine

Professor of Surgery
1991-President
Washington University School of Medicine

Leave of Absence
1/1/94-7/1/95

Commercial Positions:

Novocell, Inc.
Chief Scientific Officer

Neocrin Company
Chief Scientific Officer 1/1/94-7/31/99
Executive Vice President of Medical Affairs 1/1/94-7/31/99
Executive Vice President for Research 1/1/94-11/1/95
Executive Vice President, Research and Development 11/1/95-President

McDonnell Douglas Corporation 1984-1987
Contractual Research Investigator
Electrophoretic Separation of Islet Cells

Cytotherapeutics, Inc. 1989-1993
Founding Scientist
Scientific Advisory Board Member
Contractual Research Investigator

Patents:

"Islet Isolation Process"
DE191613T – 1987
DE3650662D – 1998
EP0191613 – 1986
EP0191613 – 1989
EP0191613 – 1997
JP61183226 – 1986
US4868121 – 1989
US5322790 – 1994

"Method to Isolate Clusters of Cell Sub-Types from Organs"
AU1934988 – 1989
CA1340406 – 1999
EP0382727 – 1990
EP0382727 – 1991
JP2504222T – 1990
US5079160 – 1992
WO8809667 – 1988

"Implantable Biocompatible Immunoisolatory Vehicle for Delivery of Selected Therapeutic Products"
AT156344T – 1997
AU666118 – 1996
AU682796 – 1997
AU2004192 – 1992
AU3902095 – 1996
CA2109085 – 1992
CA2109085 – 2003
DE69221484D – 1997
DE69221484T – 1998
DK585368T – 1998
EP0585368 – 1994
EP0585368 – 1994
EP0585368 – 1997
ES2107537T – 1997
FI934545 – 1993
FI934545D – 1993
GR3025301T – 1998
HK1001832 – 1998
JP6507412T – 1994
NO308198B – 2000
NO933842 – 1993
SG47470 – 1998

US5798113 – 1998
US5800828 – 1998
US5871767 – 1999
US6083523 – 2000
US6322804 – 2001
US2002150603 – 2002
WO9219195 – 1992

"Methods for Coextruding Immunoisolatory Implantable Vehicles with a Biocompatible Jacket and a Biocompatible Matrix Core"
US5800829 – 1998

"Methods for Treating Diabetes by Delivering Insulin from Biocompatible Cell Containing Devices"

US5869077 – 1999

"Methods for Making Immunoisolatory Implantable Vehicles with a Biocompatible Jacket and a Biocompatible Matrix Core"
US5834001 – 1998
US5874099 –1999

"Use of Pouch for Implantation of Living Cells"
AU4788993 – 1994
CA2140905 – 1994
EP0655910 – 1995
EP0655910 – 1996
JP8500033T – 1996
US5916554 – 1999
WO9403154 – 1994

Hospital Appointments:

Assistant Surgeon 1976-1983
Associate Surgeon - 1983-Present
Barnes Hospital, St. Louis, Missouri

Attending Surgeon 1982-1985
Consulting Surgeon 1976-1982
Associate Chief of Surgery 12/86-9/90
Veterans Administration Medical Center, St. Louis, Missouri

Consulting Surgeon 1985-7/95
Acting Chief of Surgery 3/86-12/86
St. Louis Regional Hospital, St. Louis, Missouri

Consulting Surgeon 1976-1985
St. Louis Children's Hospital, St. Louis, Missouri

Consulting Surgeon 1976-1985
Chief of Surgery 1981-1982
St. Louis County Hospital, St. Louis, Missouri

Attending Surgeon 1976-1982
St. Louis City Hospital, St. Louis, Missouri

Licensure: Missouri 1970

Certification: American Board of Surgery 1979
Fellow American College of Surgery 1982
Recertification: American Board of Surgery 1989

Medical Societies:

American College of Surgeons
American Diabetes Association
American Federation of Clinical Research
American Pancreatic Association
American Society for Artificial Internal Organs
American Surgical Association
Association for Academic Surgery
Society of University Surgeons
Tissue Culture Association
American Society of Transplant Surgeons
The Cell Transplantation Society
United Network of Organ Sharing - Region 8
International Pancreas & Islet Transplantation Association

Honors and Awards:

St. Louis Surgical Society Award for Research
Recipient 1973 & 1974
NIH Research Career Development Award
Recipient 1977-1982
NIH NAIMMDD Site Visit Teams
Member 1980-1995

NIH Surgery, Anesthesiology, and Trauma Study Section
Member 1985-1989
Reserve Member Status 1989-1995
World Journal of Surgery
Guest Editor - Islet Transplantation Symposium 1984
"Separation of Islet Cells in Microgravity by Continuous-Flow Electrophoresis", NASA - McDonnell Douglas Astronautics Corp. - STS-8, Space Shuttle, "Challenger", Experiment 1984

Editorial Reviewer:
Diabetes, Surgery, Journal Clinical Investigation
Grant Reviewer
Canadian Diabetes Association
Medical Research Council of Canada
National Surgical Advisor - Digestive Disease Center of Excellence - The Humana Corporation 1986-1994
Alpha Omega Alpha - Washington University Chapter - Elected
Faculty Member January 1988
Outstanding Profession/Scientific Employee - Federal Employee of the Year Award Program - St. Louis Federal Executive Board 1990
The Huddinge Hospital Transplant Lectureship - Annual Meeting of the Swedish Society for Medical Science, Stockholm, Sweden, December 1990
Council Member - Cell Transplantation Society 1992-Present
Council Member - International Pancreas & Islet Transplantation Association 1993-Present
Editorial Board
Cell Transplantation 1992-1993
Transplantation Science

Committee Appointments:

Washington University Animal Studies Committee
Chairman 1991-1994
Washington University Medical Center Alumni Association
Committee Member 1991-1994
International Juvenile Diabetes Research Foundation Medical Science Review Committee 1990-1993
UNOS Pancreas Subcommittee Member 1991-1995
American Society of Transplant Surgeons Program and Publications Committee 1989-1991
Academic Freedom and Tenure Hearing Committee
Member 1985-1991
Washington University Committee on the Humane Care of Laboratory Animal Member
Member 1984-1990

Operating Room Technician Program
Forest Park Community College
Advisory Committee 1976-1995
Chairman 1986-1995
Mid-America Transplant Association
Member Professional Advisory Board 1985-1995
American Cancer Society Institutional Research Grants
Washington University Committee for Cancer Research
Member 1979-1989
Chairman 1982-1989
"Health Views" - Editorial Advisory Board
Member 1984-1988
American Diabetes Association, St. Louis Chapter
Research Committee 1985-1988
Department of Surgery Animal Facility
Director 1980-1984
Washington University Faculty Senate
Member 1981-1983
Executive Committee of the Faculty Council
Member 1982-1984
Clinical Sciences Research Building Animal Surgery Suite
Director 1984-1985
Department of Engineering Master Degree Thesis Review Committee:
1979-John Bergen - "Kinetics of Insulin Secretion from Pancreatic Islets of Langerhans and Development of Islet Transplantation Chambers"
1980-Paul Aegerter - "Microencapsulation of Living Cells to Prevent Immunological Response"
1983-Shiow Meei Lin - "Testing of a Mathematical Model for Islet Transplantation Chambers"
1987-Donna Wilkinson - "Coating of Live Cells with Polysaccharide Derivatives"
1989-Mary Blanchard - "Quantification of Low Concentrations of Polysaccharide Derivatives and Their Effect on Cell Viability"
1990-Ph.D. Thesis Review, Donna Hawk-Reinhard - "Purification of Pancreatic Islets of Langerhans Using Cell Electrophoresis"
St. Louis VAMC Committees
Comprehensive Planning Committee
Chairman 1988-1990
Administrative Executive Board 1988-1990
Professional Standards Board 1988-1990
Research Committee 1988-1994
District Planning Board 1988-1990

Barnes Hospital Committees
- Chaplaincy Committee 1992-1994
- Emergency Room Committee 1978-1984
- Search Committee for ER Director 1978-1984
- Patient Education Parent Committee 1979
- Surgery Patient Education Subcommittee Chairman 1981-1988

Tissue Culture Association
- Publicity Chairman 1980

Invited Presentations, Selected:

The Kroc Foundation
- Islet Transplantation Workshop 1974
- Islet Transplantation Workshop 1979
- Islet Transplantation Workshop 1982

National Institutes of Health
- National Conference on Diabetes 1979
- National Conference on Diabetes 1983

Juvenile Diabetes Foundation
- Conference on Research Tissue 1981
- National Meeting, Keynote Speaker 1984
- International Scientific Research Conference 1985

German Diabetes Association, Giessen, West Germany
- Islet Transplantation Workshop 1980
- Islet Transplantation Workshop 1989

American Society of Artificial Organs
- Annual Meeting - Keynote Speaker 1983
- Session Co-Chairman 1987

International Symposium on Organ Transplantation in Diabetes
- The Hague, Netherlands 1983

International Symposium on Kidney and Pancreas Transplantation
- Perugia, Italy 1984

International Islet Transplantation Workshop
- Canberra, Australia 1984

XII Congress of the International Diabetes Federation
- Madrid, Spain 1985

XIII Congress of the International Diabetes Federation
- Sydney, Australia 1989

National Disease Research Interchange
- Human Tissue Conference 1985
- Human Tissue Conference 1986
- Human Tissue Conference 1987
- Human Tissue Conference 1990

National Disease Research Interchange - Chairman of Task Force on "Biohazard and Contamination in the Use of Human Tissue and Organs"
Philadelphia, PA 1988

American Diabetes Association National Meeting - Session Co-Chairman for "Forms of Therapy" 1986

Visiting Scientist Program - University of Kansas Diabetes Center
Kansas City, Kansas 1986

Immunology of Diabetes Symposium - Member of International Advisory Committee
Edmonton, Canada 1986

International Symposium on Complications of Diabetes
The Hague, Netherlands 1986

Visiting Professorship - Department of Surgery - University of Minnesota
Minneapolis, Minnesota 1986

May 8th Endocrine Days
Victoria, British Columbia 1987

Second Annual Visiting Professorship in Diabetes - University of Wisconsin
Madison, Wisconsin 1987

First International Course on Transplantation
Venice, Italy 1987

Progress in Organ Transplants, Tissue Replacements and Implants
Sponsored by Biomedical Business International, New York 1987

Josiah Brown Memorial Symposium on Pancreas Beta Cell Transplantation
Los Angeles, California 1987

Seventh Workshop of the AIDSPIT Study Group
Igls, Austria 1988

First international Congress on Pancreatic and Islet Transplantation
Stockholm, Sweden 1987

Thirty-Fourth Annual Meeting of ASAIO, Invited Speaker "Modern Treatment of Insulin Dependent Diabetes"
Reno, Nevada 1988

Sixth Gordon Research Conference on Drug Carriers in Biology and Medicine
Plymouth, New Hampshire 1988

XII International Congress of the Transplantation Society
Sydney, Australia 1988

Second International Congress on Pancreatic and Islet Transplantation
Minneapolis, Minnesota 1989

Biology of Tissue Transplantation Symposium
Bethesda, Maryland 1989

Ninth Workshop of the AIDSPIT Study Group
Igls, Austria 1990

Society for Surgery of the Alimentary Tract Postgraduate Course, "Medical Aspects of Transplantation of the Liver, Pancreas and Intestine"
San Antonio, Texas 1990

Moderator for Pancreas Transplantation Scientific Session - American Society of Transplant Surgeons
Chicago 1990
UCLA Symposium on Molecular & Cellular Biology, "Tissue Engineering"
Keystone, Colorado 1990
The Huddinge Hospital Transplant Lectureship Annual Meeting of the Swedish Society for Medical Science
Stockholm, Sweden 1990
Third International Congress on Pancreatic and Islet Transplantation - Moderator and Plenary Speaker
Lyon, France 1991
European Association for the Study of Diabetes - Plenary Speaker
Dublin, Ireland 1991
Visiting Professor - University of Wisconsin
Madison, Wisconsin 1991
Moderator for Clinical Transplantation-Pancreas and Islets - XVIth International Congress of the Transplantation Society
Paris 1992
American Diabetes Association 53rd Annual Meeting - Plenary Speaker
Las Vegas, Nevada 1993
Fourth International Congress of Pancreas and Islet Transplantation - Plenary Speaker
Amsterdam 1993
IVth Joint Meeting of the Lawson Wilkins Pediatric Endocrine Society and the European Society for Pediatric Endocrinology - Symposium Speaker
San Francisco 1993
American Association for Clinical Chemistry
New York 1993

Publications

Abstracts:

1. Ballinger, W.F., Lacy, P.E., Scharp, D.W. Kemp, C.B., Knight, M. - Isografts and allografts of pancreatic islets in rats. Brit. J. Surg. 60:313, 1973

2. Kemp, C.B., Knight, M.J., Scharp, D.W., Lacy, P.E., Ballinger, W.F. - Islets of Langerhans injected into the portal vein of the diabetic rat. South African Journal of Surgery 11:135, 1973

3. Kemp, C.B., Knight, M.J., Scharp, D.W., Lacy, P.E., Ballinger, W.F. - Proceedings: Implantation of pancreatic islets into the portal vein of diabetic rats. Brit. J. Surg. 60:907, 1973

4. Scharp, D.W., Kemp, C.B., Knight, M.J., Murphy, J., Newton, W., Ballinger, W.F., Lacy, P.E. - Long term results of portal vein islet isografts and allografts in the treatment of Streptozotocin induced diabetes. Diabetes 23:359, 1974

5. Scharp, D.W., White, D.J., Ballinger, W.F., Lacy, P.E. - Transplantation of intact islets of Langerhans after tissue culture. In Vitro 9:364, 1974

6. Knight, M.J., Scharp, D.W., Kemp, C.B., Nunnelley, S.B., Ballinger, W.F., Lacy, P.E. - Cryopreservation of pancreatic islets. European Surgical Research 6(1):89, 1974

7. Ballinger, W.F., Murphy, J.J., Scharp, D.W., Hirshberg, G.E., Karl, R.C., Lacy, P.E. - Isolation and preservation of human islets of Langerhans for transplantation in the treatment of diabetes. European Society for Exp. Surg., Tenth Congress 1975

8. Griffith, R.C., Scharp, D.W., Ballinger, W.F., Lacy, P.E. - A morphologic study of intrahepatic portal vein islet isografts. Diabetes 34(2):419, 1975

9. Dodi, G., Scharp, D., Feldman, S., Maresca, B., Ballinger, W., Lacy, P. - Treatment of exocrine pancreatic dysfunction in diabetic rats by islet transplantation. European Surgical Research 9(1):98, 1977

10. Scharp, D.W., Merrell, R.C., Feldman, S., Ruwe, E., Feldmeier, M., Ballinger, W., Lacy, P. - Long term culture of islets of Langerhans utilizing a rotational culture method. In Vitro 13:174, 1977

11. Scharp, D., Krupin, T., Waltman, S., Oestrich, C., Feldman, S., Ballinger, W., Becker, B - Relationship of abnormal insulin release to fluorophotometry in experimental diabetes. Diabetes 27(2):435, 1978

12. Scharp, D.W., Merrell, R.C., Feldmeier, M.M., Downing, R., Ballinger, W.F. - Pseudo-islet formation and culture from canine isolated pancreatic cells. In Vitro 15:216, 1979

13. Rajotte, R.V., Scharp, D.W., Downing, R. Molnar, G.D., Ballinger, W.F. - The transplantation of frozen-thawed rate islets transported between centers. Diabetes 28:377, 1979

14. Downing, R. Scharp, D.W., Grieder, M., Ballinger, W.F. - Mass isolation of islets of Langerhans from the dog pancreas. Diabetes 28:426, 1979

15. Feldman, S.D., Scharp, D.W., Lacy, P.E., Ballinger, W.F. - Fetal pancreas isografts, cultured and uncultured to reverse Streptozotocin induced diabetes mellitus. The Association for Academic Surgery 12:116, 1979

16. Grieder, M.H., DeSchryver-Kecskemeti, K. Gingerich, R.L., Scharp, D.W. - In vitro studies using canine pseudo-islets and rat antrum cultures as models. UCLA Symposium, December 3, 1979

17. Scharp, D.W., Feldmeier, M.M., Rajotte, R.V., DeSchryver, K., Bell, M. - Human pseudo-islet formation, culture and preservation. Diabetes 29(2):18A, 1980

18. Gingerich, R.L., Scharp, D.W., Grieder, M.H., Dye, E.S., Mousel, K.A. - A new in vitro model to study secretion and biochemistry of pancreatic polypeptide (PP). Diabetes 29(2):30A, 1980

19. Bergen, J.F., Mason, N.S., Scharp, D.W., Sparks, R.E. - Insulin inhibition of islets in transplantation chambers. Presented at International Society for Artificial Organs Meetings, Paris, July 8-10, 1981

20. Sparks, R.E., Mason, N.S., Finley, T.C., Scharp, D.W. - Development, testing and modeling of an islet transplantation chamber, ASAIO Meetings, Chicago, April, 1982

21. Long, J.A., Adair, W.S., Scharp, D.W. - Hybridoma production against pancreatic cells. Diabetes 31(2):20A, 1982

22. Scharp, D.W., Hirshberg, G., Long, J.A. - The effect of islet dosage and time on rat portal vein isografts. Diabetes 31(2):162A, 1982

23. Scharp, D.W., Lacy, P.E. - The isolation and alteration of islet tissue for transplantation. The Tissue Culture Association Meeting, San Diego, June, 1982. In Vitro Suppl. 1, 1982

24. Long, J.A., Adair, W.S., Scharp, D.W. - An immunological approach to islet cell purification. J. Cell Biol. 95:4061, 1982

25. Sparks, R.E. Mason, N.S., Finley, T.C., Scharp, D.W. - Design of islet transplantation chambers giving a normal glucose tolerance test. ISAO Meetings, Kyoto, Japan, November, 1983

26. Sparks, R.E., Mason, N.E., Finley, T.C., Scharp, D.W. - Islet transplantation chamber models - assumption for insulin generation and glucose diffusion. International Symposium on Organ Transplantation in Diabetes, The Netherlands, September, 1983

27. Sparks, R.E., Mason, N.S., Scharp, D.W. - Some present directions in research on tissue transplantation chambers. International Conference on Artificial Organs, Glasgow, Scotland, September, 1983

28. Sparks, R.E., Mason, N.S., Finley, T.C., Scharp, D.W. - "A distributed source-model for hybrid artificial pancreas", presented by ASAIO, Toronto, Ontario, Canada, April, 1983

29. Scharp, D.W., Feldmeier, M.M., Olack, B.J., Swanson, C.J., O'Shaughnessey, S.F. - Electrophoretic purification of islet cells for transplantation. Diabetes 33(1):179A, 1984

30. Scharp, D.W., Rajotte, R.V., Kneteman, N.M., Lacy P.E. - Zero gravity electrophoresis of islet cells. 10th International Congress of the Transplantation Society Meeting, Minneapolis, August, 1984

31. Scharp, D.W., Lacy, P.E. - Human islet isolation and transplantation. Diabetes 34(1):5A, 1985

32. Kneteman, N.M., Alderson, D., Scharp, D.W. - Canine pancreatic islet allotransplantation: dose adjusted cyclosporine A vs azathioprine - steroid, Diabetes 34(1):62A, 1986

33. Alderson, D., Kneteman, N.M., Scharp, D.W. - The isolation of purified human islets of Langerhans. Diabetes 34(1):81A, 1986

34. Corlett, M.P., Fonseca, P., Scharp, D.W. - Detrimental effect of warm ischemia on islet isolation in rats and dogs with protection by oxygen free radical scavengers. Diabetes 36(1):223A, 1987

35. Scharp, D.W., Lacy, P.E., Finke, E.H., Olack, B.J. - Seven day culture of Ficoll purified human islets. Diabetes 36(1):222A, 1987

36. Corlett, M.P., Scharp, D.W. - Effect of warm ischemia on islet isolation in rats and dogs. The Association for Academic Surgery, Orlando, Florida, November 1-4, 1987

37. Misler, S., Gee, W., Scharp, D., Manchester, J., Falke, L. - Metabolically regulated potassium channels in human islet cells. Diabetes 37(1):6A, 1988

38. Atkinson, M.A., Maclaren, N.K., Riley, W.J., Scharp, D.W., and Holmes, L. - M_r 64,000 autoantibodies (64KA) predict insulin dependent diabetes (IDD). Diabetes 37(1):98A, 1988

39. Scharp, D.W., Lacy P.E., Ricordi, C., Boyle, P., Santiago, J.V., Gingerich, R.L., Anderson, C.B., Flye, M.W. - Human islet transplantation in patients with Type I diabetes. Presented at XII International Congress of the Transplantation Society, Sydney, Australia, August 14-19, 1988

40. Riley, W.J., Maclaren, N.K., Atkinson, M.A., Scharp, D.W., Lacy, P.E. - Islet autoantibodies in "prediabetes". Ninth International Workshop on the Immunology of Diabetes, Satellite Symposium on Beta Cell Destruction and Restoration in Type I Diabetes. November 27-29, Sydney, Australia, 1988

41. Atkinson, M.A., Maclaren, N.K., Riley, W.J., Scharp, D.W., Holmes, L. - The natural history and predictive value of M_r 64,000 islet cell antigen. Ninth International Workshop on the Immunology of Diabetes, Satellite Symposium of the International Diabetes Federation, Melbourne, Australia, 1988

42. Scharp, D.W., Chern, H.T., McCullough, C.S., Gingerich, R.L. - Lack of glucose induced insulin responsiveness in canine islets. 49th Annual Meeting of the American Diabetes Association, Detroit, Michigan, June 3-6, 1991. Diabetes 38(1):209A, 1989

43. Chern, H.T., Scharp, D.W., McCullough, C.S. - Cryogenic storage of purified canine islets. 49th Annual Meeting of the American Diabetes Association, Detroit, Michigan, June 3-6, 1989. Diabetes 38(1):246A, 1989

44. Callery, M.P., Ricordi, C., Scharp, D.W., Kamei, T., Lacy, P.E., Flye, M.W. - Hepatic insufficiency following portacaval shunting is prevented by prior intraportal pancreatic islet autotransplantation. The Society of University Surgeons, Baltimore, Maryland, 1989

45. Scharp, D.W., Corlett, M.P., Ricordi, C., Lacy, P.E. - Microbial contamination risk in human islet isolation. Study Group of the European Association for the study of Diabetes, Igls, Austria, 1989

46. Ricordi, C., Lacy, P.E., Socci, C., Finke, E.H. Dye, E., Olack, B.J., Swanson, C., Scharp, D.W. - Pancreatic islet isolation in mammals: Developments of the automated method. Study Group of the European Association for the Study of Diabetes, Igls, Austria, 1989

47. Callery, M.P., Kamei, T., Flye, M.W., Scharp, D.W. - Intrahepatic islet autografts in dogs function despite completely diverting portacaval shunt (PCS). Second International Congress on Pancreatic and Islet Transplantation, Minneapolis, Minnesota, 1989

48. McCullough, C., Chern, H.T., Scharp, D.W., Swanson, C.J., Olack, B.J., Davis, C.G. - Influence of transplant site on efficiency of canine islet cell auto-transplantation. Second International Congress on Pancreatic and Islet Transplantation, Minneapolis, Minnesota, 1989

49. Scharp, D.W. - islet Preservation - Short term for quality control testing. Presented at The Third International Conference on The Use of Human Cells, Tissues and Organs in Research. Washington, D.C., September 17-18, 1998

50. Falqui, L., Kowalski, L., Bittner, P., Olack, B.J., Scharp, D.W., Lacy, P.E. - Immunosuppressive regimens to prolong survival of islet xenografts transplanted across a wide species barrier (dog to mouse). Ninth Workshop of the AIDSPIT Study Group, Igls, Austria, January, 1990

51. Scharp, D.W., Lacy, P.E., Santiago, J.V., Boyle, P.J., Weide, L, Falqui, L., Marchetti, P, Gingerich, R.L., Cryer, P.E., Jaffe, A.S., McCullough, C.S., Anderson, C.B., Flye, M.W. - A clinical trial of islet transplantation. Ninth Workshop of the AIDSPIT Study Group, Igls, Austria, January, 1990

52. Marchetti, P., Falqui, L., Scharp, D.W., Lacy, P.E. - An improved method for large scale isolation of pure, viable islets of Langerhans from the adult porcine pancreas. Ninth Workshop of the AIDSPIT Study Group, Igls, Austria, January 1990

53. Scharp, D.W., Lacy, P.E., Santiago, J.V., McCullough, C.S., Weide, L.G., Falqui, L., Marchetti, P. Gingerich, R.L., Cryer, P.E., Jaffe, A.S., Anderson, C.B., Flye, M.W. - Preliminary results of clinical trials of human islet transplants. American Society of Transplant Surgeons 16th Annual Scientific Meeting, Chicago, Illinois, May 30-June 1, 1990

54. Marchetti, P., Scharp, D.W., Finke, E.H. - The isolation, function and xenotransplantation of porcine islets of Langerhans (PIL). 50th Annual Meeting of the American Diabetes Association, Atlanta, Georgia, June 16-19, 1990

55. Scharp, D.W., Lacy, P.E., McCullough, C.S., Weide, L., Boyle, P., Santiago, J.V., Gingerich, R., Cryer, P., Anderson, C.B., Flye, M.W. - Intraportal human islet transplants. XIII International Congress of The Transplantation Society, San Francisco, California, August 19-24, 1990

56. Finke, E.H., Marchetti, P., Swanson, C.J., Falqui, L., Lacy, P.E., Scharp, D.W. - Large scale isolation, function and transplantation of islets of Langerhans. XIII International Congress of the Transplantation Society, San Francisco, California, August 19-24, 1990

57. Olack, B.J., Swanson, C.J., McLear, M., Longwith, J., Scharp, D.W., Lacy, P.E. - Islet purification using Euroficoll gradients. XIII International Congress of the Transplantation Society, San Francisco, California, August 19-24, 1990

58. Scharp, D.W. - Clinical Trials. Presented at The Third International Conference on The Use of Human Cells, Tissues and Organs in Research. Washington, D.C., September 17-18, 1990

59. Swanson, C.J., Olack, B.J., Marchetti, P., Scharp, D.W. - Long-term metabolic and hormonal changes after intrasplenic canine islet transplantation. Fourteenth International Diabetes Federation Congress, Washington, D.C., June 23-28, 1991

60. Olack, B.J., Marchetti, P., Swanson, C.J., Kneteman, N.M., Scharp, D.W. - No adverse effects of Cyclosporine A on either oral or intravenous glucose tolerance tests in Transplanted Dogs. Fourteenth International Diabetes Federation Congress, Washington, D.C., June 23-28, 1991

61. McLear, M., Marchetti, P., Gingerich, R., Scharp, D.W. - The function of isolated human islets In Vitro. Fourteenth International Diabetes Federation Congress, Washington, D.C., June 23-28, 1991

62. Marchetti, P., Swanson, C.J., Olack, B.J., McCullough, C.S., Scharp, D.W. - The effect of islet transplant site and mass on long-term metabolic function of canine islet autografts. Fourteenth International Diabetes Federation Congress, Washington, D.C., June 23-28, 1991

63. Olack, B.J., Hill, A.C., Scharp, D.W., Lacy, P.E. - Automated purification of canine islets. Third International Congress on Pancreatic and Islet Transplantation, Lyon, France, June 6-8, 1991

64. McLear, M., Marchetti, P., Finke, E., Gerasimidi, A., Scharp, D.W., Lacy, P.E. - Porcine islet function: effects of arginine, leucine, and butyrate. Third International Congress on Pancreatic and Islet Transplantation, Lyon, France, June 6-8, 1991

65. Swanson, C.J., Marchetti, P., Newton, M., Scharp, D.W. - Effects of insulin therapy on glucose metabolism and insulin secretion in dogs and pancreatic islet autografts. Third International Congress on Pancreatic and Islet Transplantation, Lyon, France, June 6-8, 1991

66. Scharp, D.W., Lacy, P.E., Longwith, J.E. - Bacteriologic risks of human islet isolation for transplantation. Third International Congress on Pancreatic and Islet Transplantation, Lyon, France, June 6-8, 1991

67. Marchetti, P., Pfiffner, K., Scharp, D.W., Lacy, P.E., Gerasimidi, A., Finke, E.H. - Successful cryopreservation of porcine pancreatic islets (PI). Third International Congress on Pancreatic and Islet Transplantation, Lyon, France, June 6-8, 1991

68. Marchetti, P., Swanson, C.J., Finke, E.H., Gerasimidi, A., Scharp, D.W., Lacy, P.E. - In Vitro function and xenotransplantation of long-term (3 weeks) cultured porcine islets of Langerhans. First international Congress on Xenotransplantation. Minneapolis, Minnesota, August 25-28, 1991

69. Marchetti, P., Giannarelli, Scharp, D.W., di Carlo, A., McLear, M., Lacy, P.E., Navalesi, R. - Metformin potentiates glucose stimulated insulin release: a study on diabetic patients and isolated human islets. European Association for the Study of Diabetes 27th Annual Meeting, Dublin, Ireland, September 10-14, 1991

70. Scharp, D.W., Marchetti, P., Swanson, C.J., Olack, B.J., Newton, M. - The effect of implantation site and islet mass on long-term survival and metabolic function of pure canine islet autografts. European Association for the Study of Diabetes 27th Annual Meeting, Dublin, Ireland, September 10-14, 1991

71. Marchetti, P., Finke, E., Pfiffner, K., Swanson, C., Gerasimidi-Vazeou, A., Olack, B., Scharp, D.W., Lacy, P.E. - Cryogenic storage of purified porcine islets. Presented at Study Group of the European Association for the Study of Diabetes, Igls, Austria, January 26-28, 1992

72. Scharp, D.W., Olack, B.J., Pfiffner, K., Gerasimidi-Vazeou, A., Finke, E.H., Lacy, P.E. - Preserving human islets prevents their rejection as xenografts. Presented at the First International Congress of the Cell Transplant Society, Pittsburgh, Pennsylvania, May 31-June 3, 1992

73. Marchetti, P., Scharp, D.W., Olack, B.J., Swanson, C.J., Bier, D., Cobelli, C., Toffolo, G., Newton, M., Navalesi, R. - Glucose metabolism, insulin sensitivity and glucagon secretion in dogs with intraportal or intrasplenic islet autografts. Presented at the First International Congress of the Cell Transplant Society, Pittsburgh, Pennsylvania, May 31-June 3, 1992

74. Marchetti, P., Scharp, D.W., Olack, B.J., Swanson, C.J. - Prediction of islet autograft failure by fasting glucagon:insulin ratio. Presented at the XIV International Congress of the Transplantation Society, Paris, France, August 16-21, 1992

75. Flavin, K.S., Scharp, D.W., Lacy, P.E. - Pre-islet transplant evaluation of psychiatric status using the National Institute of Mental Health (NIMH) Diagnostic Interview Schedule (DIS). Presented at the American Diabetes Association Meetings, San Antonio, Texas, June 20-23, 1992

76. Hawk-Reinhard, D.R., Scharp, D.W., Sparks, R.E. - Buffer solution temperature and velocity profiles in vertical micro-electrophoresis. Presented at American Institute of Chemical Engineers, New York, NY, November, 1992

77. Marchetti, P., Scharp, D.W., Finke, E., Pfiffner, K., Swanson, C., Gerasimidi-Vazeou, A., Olack, B., Navalesi, R., Lacy, P.E. - Insulin release upon perifusion and xenotransplantation of cryopreserved porcine islets. Presented at European Association for the Study of Diabetes, Igls, Austria, January 1992

78. di Carlo, A., Scharp, D.W., Gingerich, R.L., Ansara, M., Olack, B., Navalesi, R., Swanson, C. - Effect of culture and cryopreservation on insulin release from isolated, perifused human islets. Hormone and Metabolic Research 25:52, 1993

79. Marchetti, P., Scharp, D.W., Finke, E., Swanson, C.J., Gerasimidi-Vazeou, A., Olack, B.J., Navalesi, R., Lacy, P.E. - Prolongation of pig-to-mouse islet xenograft survival. Hormone and Metabolic Research 25:62, 1993

80. Hegre, O., Laurance, M., Harvey, J., Dionne, K., Appel, M., Scharp, D., Lacy, P.E. - Species variation in insulin secretory capacity of isolated islets. Hormone and Metabolic Research 25:58, 1993

81. Marchetti, P., Scharp, D.W., McLear, M., Finke, E.H., Olack, B.J., Swanson, C.J., Giannarelli, R. Navalesi, R., Lacy, P.E. - Pulsatile insulin secretion from isolated human pancreatic islets. Presented at the 53rd Annual Meeting of the American Diabetes Association Meeting, Las Vegas, Nevada, June, 1993

82. Shiroki, R., Poindexter, N., Mohanakumar, T., Scharp, D.W. - Rejection of human islet allografts in human lymphocyte reconstituted severe combined immunodeficient mice (Hu-Scid). Presented at the Fourth International Congress on Pancreas and Islet Transplantation, Amsterdam, The Netherlands, June, 1993

83. Marchetti, P., Scharp, D.W., Finke, E., Swanson, C.J., Gerasimidi-Vazeou, A., Olack, B.J., Navalesi, R., Lacy, P.E. - Effect of different temporary immunosuppressive therapies and low-temperature culture on pancreatic islet xenograft survival (pig-go-mouse). Presented at the Fourth International Congress on Pancreas and Islet Transplantation, Amsterdam, The Netherlands, June, 1993

84. Ansara, M.F., Saudek, F., Newton, M., Cryer, P.E., Scharp, D.W. - Islet transplantation prevents defective glucose counterregulation in diabetic dogs. Presented at the Fourth International Congress on Pancreas and Islet Transplantation, Amsterdam, The Netherlands, June, 1993

85. Scharp, D.W., Ansara, M.F., di Carlo, A., Flavin, K.S., White, N.H., Jendrisak, M.D., Lacy, P.E. - Intrasplenic islet transplants in kidney allograft recipients. Presented at the Fourth International Congress on Pancreas and Islet Transplantation, Amsterdam, The Netherlands, June, 1993

86. di Carlo, A., Scharp, D.W., Gingerich, R.L., Black, B.J., Swanson, C.J., Navalesi, R. - Insulin and glucagon release from isolated, perifused human islets following low temperature culture and cryopreservation. Presented at the Fourth International Congress on Pancreas and Islet Transplantation, Amsterdam, The Netherlands, June, 1993

87. Atkison, P., Zucker, P, Hramiak, I., Paul, T., McCallu, J., Stiller, KC.R., Scharp, D.W., Lacy, P.E., Olack, B., Finegood, D.T. - Continued insulin dependence despite normal insulin sensitivity and graft insulin secretion in a kidney/islet transplant patient. Presented at the Fourth International Congress on Pancreas and Islet Transplantation, Amsterdam, The Netherlands, June, 1993

88. Scharp, D.W., Swanson, C.J., Olack, B.J., Latta, P.P., Hegre, O.D., Gentile, F., Lacy, P.E. - Encapsulated human islet allografts in non-diabetic, Type 1 and Type 2 diabetic recipients. Presented at The 13th Workshop of the AIDSPIT Study Group, Igls, Austria, January 23-25, 1994. Horm. Metab. Res. 26:76, 1994

89. Scharp, D.W., Ansara, M.F., Brennan, D.C., White, N.H., Flavin, K.S., Zukosky, J.M., Cryer, P.E., Lacy, P.E. - Modification in methodology results in improvement in simultaneous kidney-islet success. Presented at the XVth World Congress of The Transplantation Society, Kyoto, Japan, August 28-September 2, 1994

90. Scharp, D.W., Brennan, D.C., White, N.H., Flavin, K.S., Lacy, P.E. - Increased islet mass raises rate of insulin independence in islet transplant recipients. Presented at IPITA 1995 Congress, Miami, Florida, June 18-22, 1995

Articles:

1. Merrick, A.W., Scharp, D.W. - Electroencephalography of resting behavior in cattle, with observations on the question of sleep, Am. J. Vet. Res., 32:1893-1897, 1971

2. Knight, M.J., Scharp, D.W., Kemp, C.B., Ballinger, W.F., Lacy, P.E. - Effects of cold storage on the function of isolated pancreatic islets, Cryobiology, 10:89-90, 1973

3. Kemp, C.B., Scharp, D.W., Knight, M.J., Ballinger, W.F., Lacy, P.E. - Importance of implantation site of pancreatic islet isografts in treatment of experimental diabetes, Surgical Forum 24:297-299, 1973

4. Kemp, C.B., Knight, M.J., Scharp, D.W., Lacy, P.E., Ballinger, W.F. - Transplantation of isolated pancreatic islets into the portal vein of diabetic rats, Nature 244:447, 1973

5. Scharp, D.W., Kemp, C.B., Knight, M.J., Ballinger, W.F., Lacy, P.E. - The use of Ficoll in the preparation of viable islets of Langerhans from the rat pancreas, Transplantation 16:686-689

6. Kemp, C.B., Knight, M.J., Scharp, D.W., Ballinger, W.F., Lacy, P.E. - Effect of transplantation site on the results of pancreatic islet isografts in diabetic rats, Diabetologia 9:489-491, 1973

7. Scharp, D.W., Murphy, J.J., Newton, W.T., Ballinger, W.F., Lacy, P.E. - Transplantation of islets of Langerhans in diabetic Rhesus monkeys, Surgery 77:100-105, 1975

8. Scharp, D.W., Murphy, J.J., Newton, W.T., Ballinger, W.F., Lacy, P.E. - Application of an improved isolation technique for islet transplantation in primates and rats, Transplant. Proc. 6(1):739-741, 1975

9. Feldman, S., Hirshberg, G., Dodi, G., Raizman, M., Scharp, D., Ballinger, W., Lacy, P.E. - Intrasplenic islet isografts, Surgery 82:386-394, 1977

10. Karl, R.C., Scharp, D.W., Ballinger, W.F., Lacy, P.E. - Transplantation of insulin-secreting tissues, J British Society Gastroenterology - GUT 18:1062-1072, 1977

11. Griffith, R.C., Scharp, D.W., Hartman, B.K., Ballinger, W.F., Lacy, P.E. - A morphologic study of intrahepatic portal vein islet isografts, Diabetes 26:201-214, 1977

12. Feldman, S., Scharp, D., Hirshberg, G., Dodi, G., Ballinger, W., Lacy, P.E. - Streptozotocin-induced liver tumors, Transplantation 24:152-154, 1977

13. Feldman, S., Dodi, G., Haid, K., Scharp, D., Ballinger, W., Lacy, P.E. - Artificial hybrid pancreas, Surgical Forum 28:439-441, 1977

14. Scharp, D.W., Merrell, R.C., Feldman, S.D., Downing, R., Lacy P,E., Ballinger, W. - The use of gyrotational culture for the preservation of isolated islets, Surgical Forum 29:100-102, 1978

15. Krupin, T., Waltman, S.R., Scharp, D.W., Oestrich, G., Feldman, S.D., Becker, D., Ballinger, W.F., Lacy, P.E. - Ocular fluorophotometry in experimental diabetes mellitus: The effect of pancreatic islet isografts, Investigative Ophthalmology & Visual Science 18:1185-190, 1979

16. Downing, R., Scharp, D.W., Ballinger, W.F. - An improved technique for the isolation and identification of mammalian islets of Langerhans, Transplantation 29:79-83, 1980

17. Lacy, P.E., Davie, J.M., Finke, E.H., Scharp, D.W. - Prolongation of islet allograft survival, Transplantation 27:171-174, 1979

18. Scharp, D.W., Downing, R., Merrell, R.C., Grieder, M. - Isolating the elusive islet, Diabetes 29(1):19-30, 1980

19. Downing, R., Scharp, D.W., Merrell, R.C., Grieder, M., Ballinger, W.F. - Venous versus ductal distension of the dog pancreas for isolation of islets, Surgical Forum 30:422-424, 1979

20. Merrell, R.C., Scharp, D.W., Gingerich, R., Feldmeier, M.M., Grieder, M., Downing, R. - New approaches to the separation of islet cells, Surgical Forum 30:303-305, 1979

21. Feldman, S.D., Scharp, D.W., Lacy, P.E., Ballinger, W.F. - Fetal pancreas isografts, cultured and uncultured, to reverse streptozotocin induced diabetes mellitus, J. Surgical Research 29:309-318, 1980

22. Grieder, M.H., Gingerich, R.L., Scharp, D.W. - In Vitro studies of canine pseudo-islets. UCLA Symposium - Cellular Basis of Chemical Messengers in the Digestive System", Los Angeles, January 16-18, 1980

23. Silberberg, R., Scharp, D.W., Lesker, P.A. - Biochemical studies of bone from diabetic rats and rats bearing transplanted pancreatic islets, Experimental Cell Biology 49:61-67, 1981

24. Rajotte, R.V., Scharp, D.W., Downing, R., Preston, R., Molnar, G.D., Ballinger, W.F. - Pancreatic islet banking: the transplantation of frozen-thawed rat islets transported between Centers, Cryobiology 18:357-369, 1981

25. Britt, L.D., Scharp, D.W. - Formation of pseudo-islets from neonatal pig pancreas, Surgical Forum 31:354-356, 1980

26. Britt, L.D., Stojeba, P.C., Scharp, C.R., Grieder, M.H., Scharp, D.W. - Neonatal pig pseudo-islets: A product of selective aggregation, Diabetes 30:580-583, 1981

27. Britt, L.D., Scharp, D.W., Lacy, P.E., Slavin, S. - Transplantation of islet cells across major histocompatibility barriers after total lymphoid irradiation and infusion of allogeneic bone marrow cells, Diabetes 31:36-38, 1982

28. Bergen, J., Mason, N.S., Scharp, D.W., Sparks, R.E. - Insulin inhibition of islets in transplantation chambers, Artificial Organs 5:787-791, 1981

29. Paquette, T.L., Gingerich, R., Scharp, D.W. - Altered amidation of pancreatic polypeptide in culture, Biochemistry 20:7403-7408, 1981

30. Schmidt, R.E., Scharp, D.W. - Axonal dystrophy in experimental diabetic autonomic neuropathy, Diabetes 31:761-770, 1982

31. Yasunami, Y., Lacy, P.E., Scharp, D.W., Britt, L.D. - Isolation and transplantation of islet cells from the neonatal pig pancreas, Surgical Forum 33, 359-361, 1982

32. Gingerich, R.L., Scharp, D.W., Grieder, M.H., Dye, E.S., Mousel, K.A. - A new in vitro model for studies of pancreatic polypeptide secretion and biochemistry, Regulatory Peptides 5:13-25, 1982

33. Scharp, D.W., Long, J.A. - Studies of islet isolation and transplantation, Cryobiology 20:129-137, 1983

34. Long, J.A., Britt, L.D., Olack, B.J., Scharp, D.W. - Autotransplantation of isolated canine islets, Transplant Proc. 15:1332-1338, 1983

35. Schmidt, R.E., Santiago, B.P., Olack, B.J., Scharp, D.W. - The effect of pancreatic islet transplantation and insulin therapy on experimental diabetic autonomic neuropathy, Diabetes 32:532-540, 1983

36. Giddings, S.J., Rotwein, P., Chirgwin, J.M., Scharp, D.W., Permutt, M.A. - Analysis of insulin gene expression in human pancreas, Diabetes 32:777-780, 1983

37. Scharp, D.W. - Isolation and transplantation of islet tissue, World J. of Surgery 8:143-151, 1984

38. Scharp, D.W., Mason, N.S., Sparks, R.E. - Islet immunoisolation: the use of hybrid artificial organs to prevent islet tissue rejection, World J. of Surgery 8:221-229, 1984

39. Scharp, D.W. - Clinical feasibility of islet transplantation, Transplant Proc. 16(3):820-825, 1984

40. Heiken, J.P., Balfe, D.M., Picus, D., Scharp, D.W. - Radical pancreatectomy: Postoperative evaluation by CT, Radiology 153:211-215, 1984

41. Martin, K.W., Morian, J.P., Jr., Lee, J.K.T., Scharp, D.W. - Demonstration of splenic artery pseudoaneurysm by Magnetic Resonance Imaging, J. of Computer Assisted Tomography 9(1):190-192, 1985

42. Downing, R., Morrissey, S., Kiske, D., Scharp, D.W. - Does the purity of intraportal islet isografts affect their endocrine function?, J. Surg. Research 41(1):41-46, 1986

43. Scharp, D.W., Lacy, P.E., Alderson, D., Kneteman, N.M. - The procurement and distribution of cadaver human pancreas for islet isolation and transplantation. The Use of Human Tissues and Organs for Research and Transplant: Proceedings of the Second International Conference: National Diabetes Research Interchange April 1986:61-70

44. Scharp, D.W., Alderson, D., Kneteman, N.M. - Optimization of islet preparations for clinical transplantation. Transplant Proc. 18(6):1814-1816, 1986

59. Ricordi, C., Scharp, D.W., Lacy, P.E. - Mass isolation of human pancreatic islets for transplantation. Proceedings from XXVI World Congress of the International College of Surgeons, Milan, Italy, July 3-9, 1988, Monduzzi Editore S.p.A.

60. Corlett, M.P., Scharp, D.W. - The effect of pancreatic warm ischemia on islet isolation in rats and dogs, J. Surg. Research, 45:531-536, 1988

61. Ricordi, C., Kneteman, N.M., Scharp, D.W., Lacy, P.E. - Transplantation of cryopreserved human pancreatic islets into diabetic nude mice, World J. Surgery 12:861-865, 1988

62. Misler, S., Gee, W.M., Gillis, K.D., Scharp, D.W., Falke, L.C. - A metabolite-regulated ATP-sensitive K+ channel in human pancreatic islet cells, Diabetes 38:422-427, 1989

63. Scharp, D.W., Lacy, P.E., Ricordi, C., Boyle, P., Santiago, J., Cryer, P., Gingerich, R., Jaffe, A., Anderson, C.B., Flye, M.W. - Human islet transplantation in patients with Type 1 diabetes, Transplant Proc 21 (No. 1):2744-2745, February 1989

64. Scharp, D.W. - Islet Transplantation, Diabetes 38: (Suppl. 1):323-325, 1989

65. Ricordi, C., Lacy, P.E., Scharp, D.W. - Automated islet isolation from human pancreas, Diabetes 38 (Suppl. 1):140-142, 1989

66. Kneteman, N.M., Alderson, D., Scharp, D.W., Lacy, P.E. - Prolonged cyropreservation of purified human pancreatic islets, Diabetes 38:176-178, 1989

67. Kneteman, N.M., Alderson, D., Scharp, D.W., Lacy, P.E. - Long-term cryogenic storage of purified adult human islets of Langerhans, Diabetes 38:386-396, 1989

68. Brelje, T.C., Scharp, D.W., Sorenson, R.L. - Three-dimensional imaging of intact, isolated islets of Langerhans using Confocal microscopy, Diabetes 38:808-814, 1989

69. Schmidt, R.E., Plurad, S.B., Olack, B.J., Scharp, D.W. - The effect of pancreatic islet transplantation and insulin therapy on neuroaxonal dystrophy in sympathetic autonomic ganglia of chronic Streptozotocin diabetic rats, Brian Research 497:393-398, 1989

70. Callery, M.P., Ricordi, C., Scharp, D.W., Kamei, T., Swanson, C.J., Lacy, P.E., Flye, M.W. - Hepatic insufficiency following portacaval shunting is prevented by prior intraportal pancreatic islet autotransplantation. Surgery 106:257-266, 1989

71. Dotta, F., Colman, P.G., Nayak, R.C., Lombardi, D., Scharp, D.W., Andreani, D., Pontieri, G.M., di Mario, U., Lenti, L., Eisenbarth, G.S. - Ganglioside expression in human pancreatic islets, Diabetes 38:1478-1483, 1989

72. Permutt, M.A., Koranyi, L., Keller, K., Lacy, P.E., Scharp, D.W., Mueckler, M. - Cloning and functional expression of a human pancreatic islet glucose-transporter cDNA, Proc Natl Acad Sci USA 86:8688-8692, 1989

73. Callery, M.P., Kamei, T., Flye, M.W., Davis, C.G., Swanson, C.J., Olack, B.J., Lacy, P.E., Scharp, D.W. - Intrahepatic islet autografts in dogs function despite completely diverting portacaval shunt, Transplant Proc 22:767-770, 190

74. Scharp, D.W., Lacy, P.E., Santiago, J.V., McCullough, C.S., Weide, L.G., Falqui, L., Marchetti, P., Gingerich, R.L., Jaffe, A.S., Cryer, P.E., Anderson, C.B., Flye, M.W. - Insulin independence after islet transplantation into Type 1 diabetic patient, Diabetes 39:515-518, 1990

75. Atkinson, M.A., Maclaren, N.K., Riley, W.J., Scharp, D.W., Lacy, P.E. - 64,000 M_r autoantibodies as predictors of insulin dependent diabetes, Lancet 335:1357-1360, 1990

76. Scharp, D.W., Olack, B.J., Swanson, C.J., Marchetti, P. - Normal reproductive function in dogs with islet autograft, Transplant Proc 22(5):2403-2404, 1990

77. Chern, H.T., Scharp, D.W. - Viability assessment of cryopreserved canine islets in vivo, Endocrine Surgery 7(3):355-360, 1990

78. Medoff, G., Sagal, S., Krogstad, D., Peters, M., Hogan, J., Scharp, D. - Clinical Pathology Conference - Abdominal pain, fever, and weight loss in a 61 year-old Arabian Man, Am J Medicine 89:83-90, 1990

79. Ricordi, C., Gray, D.W.R., Hering, B.J., et al - Islet isolation assessment in man and large animals, Acta Diabetoal. Lat. 27:185-195, 1990

80. Atkinson, M.A., Maclaren, N.K., Holmes, L., Scharp, D.W., Lacy, P.E., Riley, W.J. - The natural history and predictive value of M_r 64,000 autoantibodies for insulin-dependent diabetes mellitus, J Autoimmunity 3:41-45, 1990

81. Atkinson, M.A., Holmes, L.H., Scharp, D.W., Lacy, P.E., Maclaren, N.K. - No evidence for serological autoimmunity to islet cell heat shock proteins in insulin dependent diabetes, J Clin Invest 87:721-724, 1991

82. Scharp, D.W., Lacy, P.E., Santiago, J.V., McCullough, C.S., Weide, L.G., Boyle, P.J., Falqui, L., Marchetti, P., Ricordi, C., Gingerich, R.L., Jaffe, A.S., Cryer, P.E., Hanto, D.W., Anderson, C.B., Flye, M.W., - Results of our first nine intraportal islet allografts in Type 1, insulin-dependent diabetic patients, Transplantation 51:76-85, 1991

83. Finke, E., Marchetti, P., Falqui, L., Swanson, C., McLear, M., Olack, B., Scharp, D.W., Lacy, P.E. - Large scale isolation, function and transplantation of islets of Langerhans from the adult pig pancreas, Transplant Proc 23(1):772-773, 1991

84. Olack, B., Swanson, C., McLear, M., Longwith, J., Scharp, D.W., Lacy, P.E. - Islet purification using Euroficoll gradients, Transplant Proc 23(1):774-776, 1991

85. Scharp, D.W., Lacy, P.E., Weide, L.G., Marchetti, P., McCullough, C.S., Flavin, K., Santiago, J.V. - Intraportal islet allografts: the use of a simulation index to represent functional results, Transplant Proc 23(1):796-798, 1991

86. Falqui, L., Finke, E.H., Carel, J.C., Scharp, D.W., Lacy, P.E. - Marked prolongation of human islet xenograft survival (human to mouse) by low-temperature culture and temporary immunosuppression with human and mouse anti-lymphocyte sera, Transplantation 51:1322-1324, 1991

87. Scharp, D.W., Lacy P.E. - The clinical feasibility of human islet transplantation, Clinical Diabetes 42-45, May/June, 1991

88. Marchetti, P., Finke, E.H., Gerasimidi-Vazeou, A., Falqui, L., Scharp, D.W., Lacy P.E.- Automated large scale isolation, *in vitro* function and xenotransplantation of porcine islets of Langerhans, Transplantation 52:209-213, 1991

89. Kowalski, L., Falqui, L., Lacy, P.E., Scharp, D.W. - Production of marked prolongation of survival of canine islet xenografts in mice by antilymphocyte sera and L3T4 antibody, Transplantation 52:1094-1097, 1991

90. Marchetti, P., Swanson, C., McLear, M., Finke, E.H., Gerasimidi-Vazeou, A., Scharp, D.W., Lacy, P.E. - In vitro function and xenotransplantation of long-term (3 weeks) cultured porcine islets of Langerhans, Transplant Proc 24(2):637, 1992

91. Kneteman, N.M., Marchetti, P., Tordjman, K., Bier, D.M., Santiago, J.V., Swanson, C.J., Olack, B.J., Scharp, D.W. - Effect of cyclosporine on insulin secretion and insulin sensitivity in dogs with intrasplenic islet autotransplants, Surgery 111(4):430-437, 1992

92. Scharp, D.W., Marchetti, P., Swanson, C.J., Newton, M., McCullough, C.S., Olack, B.J. - The effect of transplantation site and islet mass on long-term survival and metabolic and hormonal function of canine purified islet autografts, Cell Transplantation 1:245-254, 1992

93. Misler, S. Barnett, D.W., Pressel, D.M., Gillis, K.D., Scharp, D.W., Falke, L.C. - Stimulus-secretion coupling in B cells of transplantable human islets of Langerhans: evidence for a critical role for Ca^{2+} entry, Diabetes 41:662-670, 1992

94. Marchetti, P., Scharp, D.W., Longwith, J.E., Swanson, C.J., Olack, B.J., Gerasimidi-Vazeou, A., Finke, E.H., Lacy, P.E. - Prevention of contamination of isolated porcine islets of Langerhans, Transplantation 53(6):1364-1366, 1992

95. Olack, B.J., Hill, A., Scharp, D.W., Lacy, P.E. - Automated purification of canine islets, Transplant Proc 24(3):1003-1004, 1992

96. Marchetti, P., Pfiffner, K., Scharp, D.W., Lacy, P.E., Gerasimidi-Vazeou, A., Finke, E.H. - Successful cryopreservation of porcine pancreatic islets, Transplant Proc 24(3):1005-1006, 1992

97. Scharp, D.W., Lacy, P.E., McLear, M., Longwith, J.E., Olack, B.J. - The bioburden of 590 consecutive human pancreata for islet transplant research, Transplant Proc 24(3):974-975, 1992

98. McLear, M., Marchetti, P., Finke, E., Gerasimidi-Vazeou, A., Scharp, D.W., Lacy, P.E. - Porcine islet function: Effects of arginine, Leucine, and Butyrate, Transplant Proc 24(3):980-982, 1992

99. Marchetti, P., Finke, E.H., Swanson, C., Gerasimidi-Vazeou, A., Olack, B., Scharp, D.W., Navalesi, R., Lacy, P.E. - The potential of porcine islet xenotransplantation in the therapy of diabetes, Diab. Nutr. Metab. 5 (Suppl 1):151-154, 1992

100. Marchetti, P., Scharp, D.W., Olack, B.J., Swanson, C.J., Bier, D., Colelli, C., Toffolo, G., Newton, M., Navalesi, R. - Glucose metabolism, insulin sensitivity and glucagon secretion in dogs with intraportal or intrasplenic islet autografts, Transplant Proc 24(6):2828-2829, 1992

101. Marchetti, P., Olack, B., Swanson, C., Newton, M., Scharp, D.W. - Assessment of long-term (1 year) graft survival and metabolic hormonal changes after intrasplenic canine pancreatic microfragment transplantation, Diabetes & Metabolism 19:17-24, 1993

102. Barnett, D.W., Pressel, D.M., Chern, H.T., Scharp, D.W., Misler, S. - cAMP-Enhancing agents "permit" stimulus-secretion coupling in canine pancreatic islet ß-cells, J. Membrane Biol 138:113-120, 1994

103. Ansara, M.F., Saudek, F., Newton, M., Cryer, P.E., Scharp, D.W. - Pancreatic islet transplantation prevents defective glucose counter-regulation in diabetic dogs, Transplant Proc. 26(2):664-665, 1994

104. Shiroki, R., Poindexter, N., Mohanakumar, T., Scharp, D.W. - Rejection of human islet allografts in human lymphocyte-reconstituted severe combined immunodeficient mice, Transplant Proc. 26(2):716-717, 1994

105. Marchetti, P., Scharp, D.W., Finke, E., Swanson, C.J., Gerasimidi-Vazeou, A., Olack, B.J., Navalesi, R., Lacy, P.E. - Effect of different temporary immunosuppressive therapies and low-temperature culture on pancreatic islet xenograft survival (Pig-to-Mouse), Transplant Proc. 26(2):760-761, 1994

106. di Carlo, A., Scharp, D.W., Gingerich, R.L., Giannarelli, R., Ansara, M., Olack, B.J., Swanson, C.J., Navalesi, R. - Insulin and glucagon release from isolated, perifused human islets following low-temperature culture and cryopreservation, Transplant Proc. 26(2):821-822, 1994

107. Marchetti, P., Scharp, D.W., Pfiffner, K., Swanson, C.J., Finke, E.H., Olack, B.J., Gerasimidi-Vazeou, A., McLear, M., Lacy, P.E. - Cryogenic storage of isolated, purified porcine pancreatic islets, Transplantation 57:340-346, 1994

108. Marchetti, P., Kneteman, N.M., Swanson, C.J., Olack, B.J., Scharp, D.W. - Effect of cyclosporin treatment on metabolic and hormonal responses to mixed meal plus oral glucose in dogs with intrasplenic pancreatic islet autograft, Experimental Medicine, In Press

109. Marchetti, P., Scharp, D.W., McLear, M., Gingerich, R., Finke, E.H., Olack, B.J., Swanson, C.J., Giannarelli, R., Navalesi, R., Lacy, P.E. - Pulsatile insulin secretion from isolated human pancreatic islets, Diabetes 43:827-830, 1994

110. Shiroki, R., Poindexter, N.J., Woodle, E.S., Hussain, M.S., Mohanakumar, T., Scharp, D.W. - Human peripheral blood lymphocyte reconstituted severe combined immunodeficient (hu-PBL-SCID) mice: a model for human islet allograft rejection, Transplantation 58:1555-1562, 1994

111. Scharp, D.W., Swanson, C.J., Olack, B.J., Latta, P.P., Hegre, O.D., Doherty, E.J., Gentile, F.T., Flavin, K.S., Ansara, M.F., Lacy, P.E. - Protection of encapsulated human islets implanted without immunosuppression in patients with Type I and Type II diabetes and in non-diabetic controls, Diabetes 43:1167, 1994

112. Marchetti, P., Scharp, D.W., Finke, E.H., Giannarelli, R., Swanson, C.J., Olack, B.J., Lacy, P.E., Navalesi, R. - Factors affecting in vitro and in vivo function of isolated porcine islets, Transplant Proc. 26(6):3419-3420, 1994

113. di Carlo, A., Scharp, D.W., Gingerich, R.L., Ansara, M.F., Olack, B.J., Swanson, C.J., Giannarelli, R., Navalesi, R. - C-peptide secretion from isolated perifused human islets: effect of culture and cryopreservation, Transplant Proc. 26(6) 3399, 1994

114. Perdrizet, G.A., Rewinski, M.J., Schweizer, R.T., Scharp, D.W. - Heat shock and recovery protects pancreatic islets from warm ischemic injury, Transplant Proc. 26(6) 3477-3478, 1994

115. Marchetti, P., Scharp, D.W., Finke, E., Swanson, C.J., Gerasimidi-Vazeou, A., Olack, B.J., Navalesi, R., Lacy, P.E. - Anti-CD4 antibody treatment in xenografts of differently immunomodulated porcine islets, Transplant Proc. 26(3):1132, 1994

116. Brennan, D.C., Scharp, D.W., White, N.H., Flavin, K.S., Zukosky, J.M., Ansara, M.F., Cryer, P.E., Lacy, P.E. - Modification of methodology results in improvements in simultaneous kidney-islet success, Transplant Proc. 27(1):1349-1350, 1995

117. Chern, H.T., Scharp, D.W. - Successful long-term cryopreservation of highly purified canine islets, European Surg Res 27:167-175, 1995

118. Marchetti, P., Scharp, D.W., Finke, E.H., Swanson, C.J., Olack, B.J., Gerasimidi-Vazeou, A., Giannarelli, R., Navalesi, R., Lacy, P.E. - Prolonged survival of discordant porcine islet xenografts, Transplantation 61:1100-1102, 1996

119. Hill, R.S., Cruise, G.M., Hager, S.R., Lamberti, F.V., Yu, X., Garufis, C.L., Yu, Y., Mundwiler, K.E., Cole, J.F., Hubbell, J.A., Hegre, O.D., Scharp, D.W. - Immunoisolation of adult porcine islets for the treatment of diabetes mellitus, Annals of the NY Academy of Sciences 831:332-343, 1997

120. Cruise, G.M., Hegre, O.D., Scharp, D,W., Hubbell J.A., - A sensitivity study of the key parameters in the interfacial photopolymerization of poly(ethylene glycol) diacrylate upon porcine islets, Biotech. Bioeng. 57:655-665, 1998

121. Cruise, G.M., Scharp, D.W., Hubbell, J.A. - Characterization of permeability and network structure of interfacially photopolymerized poly(ethylene glycol) diacrylate hydrogels, Biomaterials 19:1287-1294, 1998

122. Cruise, G.M., Hegre, O.D., Lamberti, F.V., Hager, S.R., Hill, R., Scharp, D.W., Hubbell, J.A. - *In vitro* and *in vivo* performance of porcine islets encapsulated in interfacially photopolymerized poly(ethylene glycol) diacrylate membranes, Cell Transplantation, 8:293-306, 1999

Letters, Reviews and Chapters:

1. Karl, R., Scharp, D.W., Ballinger, W.F., Lacy, P.E. - Progress Report: transplantation of insulin secreting tissue, Gut. 18:1062-1072, 1977

2. Lacy, P.E., Scharp, D.W., Ballinger, W.F. - Transplantation of the islets of Langerhans - future directions. 14th International Congress of Therapeutics, p. 105, L'Expansion Scientique Francaise, Montpellier, France, 1977

3. Scharp, D.W. - Rejection of islet "isografts" in a strain of Lewis rats, Transplantation, 31:229-230, 1981

4. Rajotte, R.V., Scharp, D.W., Molnar, G.D., Preston, R. - Feasibility of low temperature banking for pancreatic islet cell transplantation. In "Islet Isolation, Culture, and Cryopreservation", Ed. K. Federlin and R.G. Bretzel. Thieme-Stratton, Inc., p. 124-135, 1981

5. Scharp, D.W., Downing, R., Merrell, R.C., Nunnelly, S., Kiske, D., Grieder, M.H. - New approaches in the methods for isolating mammalian islets of Langerhans utilizing the dog pancreas. In "Islet Isolation, Culture, and Cryopreservation". Ed. K. Federlin and R.G. Bretzel. Thieme-Stratton, Inc. p. 9-31, 1981

6. Slavin, S., Britt, L.D., Scharp, D.W., Lacy, P.E. - Treatment of experimental diabetes with histoincompatible islet allografts in rats conditioned with total lymphoid irradiation. In "Diabetic Renal-Retinal Syndrome", Volume 2. Ed. E.A. Friedman and E.A. L'Experance. Grune and Stratton, Inc., NY, p. 519-528, 1982

7. Chen, Y., Mason, N.S., Scharp, D.W., Ballinger, W.F., Sparks, R.E. - Collagenase immobilized on cellulose acetate membranes. In "Biomaterials: Interfacial Phenomena and Applications". Ed. S.L. Cooper and N.A. Peppas, American Chemical Society, Washington, D.C., p. 483-491, 1982

8. Scharp, D.W. - Hybrid Artificial Pancreas Panel Discussion. Transactions of the American Society for Artificial Internal Organs, 28:642-643, 1982

9. Scharp, D.W. - Clinical Feasibility of Islet Transplantation, International Symposium on Organ Transplantation in Diabetes, The Netherlands 1983, Transplant Proc. 16:820-825, 1984

10. Scharp, D.W., Mason, N.S., Sparks, R.E. - The use of hybrid artificial organs to provide immunoisolation to endocrine grafts. In "Bone Marrow & Organ Transplantation", Ed. S. Slavin, Elsevier Science Publishers, New York, p. 601-618, 1984

11. Scharp, D.W., Long, J.A., Feldmeier, M.M., Olack, B.J., O'Shaughnessy, S., Swanson, C.J. - Automated methods for isolating and purifying islet cells from the pancreas. In "Methods in Diabetes Research", Ed. Larner and Pohl, New York, John Wiley & Sons, p. 237-243, 1984

12. Scharp, D.W., Long, J.A., Feldmeier, M.M., Olack, B.J., O'Shaughnessy, S., Swanson, C.J. - Automated methods of mass islet isolation. In "Methods in Diabetes Research", Ed. Larner and Pohl, New York, John Wiley & Sons, p. 225-236, 1984

13. Slavin, S., Weiss, L., Weigensberg, M., Morecki, S., Fuks, Z., Britt, L., Lacy, P.E., Scharp, D.W. - Tollerance to alloantigens in rodents and reversal of streptozotocin-induced diabetes in tolerant rats by histo-incompatible islet allografts. In "Bone Marrow & Organ Transplantation", Ed. S. Slavin, Elsevier Science Publications, New York, 1984

14. Williamson, J.R., Olack, B.., Rowald, E.A., Chang, K., Scharp, D.W. - Effects of insulin treatment on collagen crosslinking in streptozotocin diabetic rats. In "Lessons from Animal Diabetes", Eds. E. Shafrir and A. Renold, John Libbey, London, 1984, p. 402-404

15. Lacy, P.E., Scharp, D.W. - Islet Transplantation in treating diabetes, Ann. Rev. Med,. 37:33-40, 1986

16. Scharp, D.W. - The elusive human islet: Variables involved in its effective recovery. In "Transplantation of the Endocrine Pancreas in Diabetes Mellitus", Eds. R. van Schifgaarde and M.A. Hardy, Elsevier Science Publications, Amsterdam, 1988, p. 97-107

17. Scharp, D.W., Lacy, P.E. - Islet transplantation: a review of the objective, the concepts, the problems, the progress and the future. In "Transplantation of the pancreas", Eds. J.M. Dubernard and D.E.R. Sutherland, Kluwer Academic Publishers, The Netherlands, 1989, p. 455-478

18. Scharp, D.W., Lacy, P.E. - Clinical islet transplantation. In "Excerpta Medica International Congress Series", Eds. R. Larkins, P. Zimmet, D. Chisholm, Elsevier Science Publishers, Amsterdam, 1989, p. 721-724

19. McCullough, C.S., Scharp, D.W., Lacy, P.E. - Human islet isolation and transplantation. In "Immunologische and Dyamische Aspekte der Insulinsubstitution beim Diabetes Mellitus", Ed. R.G. Bretzel, Springer-Verlag, Heidelberg, 1990, p. 260-267

20. Atkinson, M.A., Maclaren, N.K., Scharp, D.W. - No role for 65KD heatshock protein in diabetes, (Letter) Lancet 336:1250-1251, 1990

21. Lacy, P.E., Scharp, D.W. - Islet cell transplantation. In "Diabetes Mellitus", Fourth Edition, Eds. H. Rifkin and D. Porte, Jr., Elsevier Science Publications, New York, 1990, P. 880-886

22. McCullough, C.S., Scharp, D.W. - Pancreas and islet transplantation for the treatment of Type I diabetes mellitus. In "Surgical Management of the Diabetic Patient", Eds. M. Bergman and G.A. Sicard, Raven Press, Ltd., New York, 1991, p. 349-362

23. Scharp, D.W. - Islet Transplantation: Approaches to a Clinical Therapy. In "Perspectives in General Surgery", Vol 3, No. 1, Ed. Barry A. Levine, Quality Medical Publishers, St. Louis, MO, 1992, p. 93-105

24. Scharp, D.W. - Islet quality control testing and the islet isolation laboratory. In "Pancreatic Islet Cell Transplantation", Ed. Camillo Ricordi, R.G. Landes Company, Austin, 1992, p. 82-88

25. Scharp, D.W. - Clinical islet transplantation - registry report, accomplishments in the past and future research needs, Invited Commentary, Cell Transplantation 2:299, 1993

26. Scharp, D.W. - Pancreatic Islet Transplantation. In "Atlas of Organ Transplantation, Ed. M. Wayne Flye, W.B. Saunders Company, Philadelphia, PA, 1994, p. 209-220

27. Ansara, M.F., Cryer, P.E., Scharp, D.W. - Perioperative Management of the Diabetic Patient. In "Care of the Surgical Patient", Ed. A. McHugh, Scientific American Medicine, New York, NY, In Press

APPENDIX 1

Non-Obese Diabetic (NOD) Mouse BAC Library

The Wellcome Trust Sanger Institute (Cambridge, United Kingdom) recently released to the scientific community a Non-Obese Diabetic (NOD) mouse BAC library containing 240,000 clones obtained from the Diabetes and Inflammation Laboratory (Cambridge, United Kingdom).

The NOD mouse, which spontaneously develops type 1 diabetes, is a valuable animal model that is used extensively in research exploring the etiology, prevention, and treatment of this disease. It is a vital research tool for testing promising prevention and treatment strategies at the preclinical level.

The Sanger Institute sequenced the complete NOD BAC library and used this resource to complete a physical map of the BAC clones. As a next step, they plan to sequence the 200,000 clones from the Pieter de Jong library (Children's Hospital, Oakland CA). These sequences will be aligned with those from the most recent version of the normal C57B1/6 (B6) mouse strain (a non-diabetic mouse strain) in an effort to identify single nucleotide differences between NOD mouse clone end sequences and the B6 mouse genome. Investigators at the Sanger Institute will use this sequencing information to identify and map candidate genes. Such information will guide efforts to isolate genes that contribute to the development of type 1 diabetes in humans.

This research was conducted as part of the Immune Tolerance Network, which is jointly funded by the National Institute of Allergy and Infectious Diseases, the National Institute of Diabetes, Digestive, and Kidney Disorders (both part of the National Institutes of Health) and the Juvenile Diabetes Research Foundation.

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APPENDIX II

◆ REVIEW ARTICLE ◆

Taylor & Francis
healthsciences

Development of new strategies to prevent type 1 diabetes: the role of animal models

Arno Hänninen[1], Emma Hamilton-Williams[2] and Christian Kurts[2,3]

Type 1 diabetes is an immune-mediated disease typically preceded by a long preclinical stage during which a growing number of islet-cell-specific autoantibodies appear in the serum. Although antigen-specific T lymphocytes and cytokines rather than these autoantibodies are the likely executors of β-cell-destruction, these autoantibodies reflect the existence of autoimmunity that targets islet β-cells. Abrogation of this autoimmunity during the preclinical stage would be the key to the prevention of type 1 diabetes. However, the quest of protecting islet-cells from the immune attack requires detailed knowledge of mechanisms that control islet-inflammation and β-cell-destruction, and of mechanisms that control immune tolerance to peripheral self-antigens in general. This knowledge can only be obtained through further innovative research in experimental animal models. In this review, we will first examine how research in non-obese diabetic mice has already led to promising new strategies of diabetes prevention now being tested in human clinical trials. Thereafter, we will discuss how recent advances in understanding the mechanisms that control immune response to peripheral self-antigens such as β-cell antigens may help to develop even more selective and effective strategies to prevent diabetes in the future.

Keywords: antigen-presentation; dendritic cells; disease prevention; immunological tolerance; non-obese diabetic mice; T-lymphocytes; transgenic animal models; type 1 diabetes

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Introduction

Type 1 diabetes (T1DM) is the clinical manifestation of the loss of insulin production in the endocrine pancreas. This is caused by immune-mediated destruction of islet β-cells. T1DM is believed to be an autoimmune disease, based on several lines of evidence (1–9). Although an infective (e.g., viral) or other environmental (e.g., dietary) agent may well be involved in the initiation of the immune attack towards islet β-cells (10, 11), it is clear that the (auto)immune attack itself is a key element in disease pathogenesis (12).

Immune responses against various islet-antigens appear even years before the clinical manifestation of T1DM, and precede the phase when sensitive metabolic tests first reveal attenuation in the function of β-cells (13). A number of islet-antigens are targeted by the immune system in T1DM, and cellular and/or humoral immune responses are detected against insulin, glutamic acid decarboxylase-65 (GAD 65), tyrosine phosphatase IA-2 and heat shock protein 60 (hsp 60) (14–16). Studies on pancreas samples obtained shortly after onset of clinical disease (as biopsies or as autopsy material following fatal ketoacidosis) have revealed the existence of cellular infiltrates in islets consisting of lymphocytes and antigen-presenting cells (APC), reflecting the immune attack and selective destruction of insulin-producing β-cells (4–6). Although studies in humans have been essential in the characterization of the disease process and its target antigens, development of novel therapeutic strategies requires studies also in experimental animal models.

The non-obese diabetic (NOD) mouse is the most widely used animal model of T1DM (17). This inbred mouse strain is unique in that it spontaneously develops autoimmune diabetes at high incidence. Although NOD mice harbour certain unique defects in their immune system (such as lack of the murine homolog of humal leukocyte antigen HLA-DR (18) and complement component C5 (19)) and have a

Abbreviations and acronyms

APC	antigen-presenting cell
CTLA-4	cytotoxic T-lymphocyte antigen-4
DC	dendritic cells
GAD65	glutamic acid decarboxylase-65
GM-CSF	granulocyte-macrophage colony-stimulating factor
HLA	human leukocyte antigen
IA-2	islet-associated-2 (tyrosine phosphatase, an autoantigen)
IL	interleukin
IFN	interferon
hsp 60	heat shock protein 60
LCMV	lymphocytic choriomeningitis virus
MHC	major histocompatibility complex
NKT	natural killer cell-like T cell
NOD	non-obese diabetic mouse
OT-I T cell	OVA-specific CD8 T cell
OVA	ovalbumin
PD-L1	programmed death ligand 1
RANK	receptor for activation of NFκB
RIP	rat insulin promoter
TGF	transforming growth factor
T1DM	type 1 diabetes mellitus
TCR	T cell receptor
TNF	tumour necrosis factor
TRAIL	TNF-related apoptosis inducing ligand
TRANCE	TNF-related activation induced cytokine
Treg	regulatory T cell

Key messages

- Type 1 diabetes (T1DM) is the clinical manifestation of immune-mediated destruction of insulin-producing β-cells which cannot be prevented yet.
- Most of the current clinical trials aiming at T1DM prevention are based on strategies developed in animal models.
- Research with these animal models has recently generated essential information regarding the cellular and molecular mechanisms regulating immune response to β-cells that can be used in the development of novel prevention strategies.

strong gender bias in diabetes incidence towards female preponderance (20), many of the important determinants are strikingly similar, including homologous susceptibility genes, major autoantigens, existence of insulitis and the ability of bone-marrow cells to transmit diabetes between individuals (21, 22).

In this review, we will first describe how research in non-obese diabetic mice has led to the discovery of new strategies of diabetes prevention that are now in human clinical trials. Thereafter, we will focus on work in transgenic animal models of islet-autoimmunity and discuss how immune tolerance towards sequestered self-antigens such as β-cell antigens is maintained, and how the tolerant state can change into a pathogenic immune response. Emphasis will be given to work elucidating the cellular and molecular basis of this balance and on therapeutic approaches that aim at posing this balance away from pathological autoimmunity.

The NOD mouse – a platform for testing existing immunomodulatory agents in the prevention of spontaneous autoimmune diabetes

Application of modern methods in molecular and cell biology to research on the mammalian immune system has allowed numerous receptor-ligand pairs and intracellular signalling pathways that regulate the function of lymphocytes and antigen-presenting cells to be identified. Through this research, an increasing number of antibodies and other reagents have become available for selective blockade or mimicking of the function of a specified molecule with a regulatory function. The NOD mouse is ideal for testing the effect on diabetes incidence of such agents. Thus, it forms a platform for identification and validation of potential drug targets and drugs in diabetes prevention.

In fact, surprisingly many different manoeuvres (over a hundred or so) have been reported to delay or diminish diabetes incidence in NOD mice (17). Many of these lack direct relevance for prevention of T1DM in humans simply because of their experimental nature. However, several potentially relevant approaches have been identified (Table 1 and references therein). As will be discussed later, some of the earliest human trials based on these approaches have now given negative outcomes that could reflect differences between T1DM in humans and the corresponding syndrome in NOD mice. However, results of other trials are more consistent with those obtained in NOD mice and support the idea that the NOD model can be used as a platform for testing various prevention protocols. Protocols applied in the NOD model have been based firstly on blocking the activation or function of T-lymphocytes or their subtypes and hence, of pathogenic effector cells, or their migration into pancreatic islets. Secondly, they have been based on administration of autoantigens in forms and schedules that are anticipated to enhance regulation of specific immune responses. Thirdly, numerous antigen-non-specific substances with immunomodulatory effects, or possible protective effect on β-cells have been applied with often favourable outcomes.

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It is conceivable that targeting a subtype of T-lymphocytes or a costimulatory pathway will affect the immune system also on a general level and induce various side effects. Therefore, there has long been a quest to develop strategies that selectively target the immune effector cells or locally inhibit tissue destruction. Accordingly, autoantigens (pro)insulin, GAD65 and hsp-60 or their peptides have been administered without immune adjuvants and/or *via* tolerogenic routes, especially intravenously or *via* mucosal surfaces (orally, intranasally or inhaled as aerosol). Results of these experiments have been promising (Table 1 and references therein) and have already led to human clinical trials, as detailed below. Unfortunately, initial results of these trials have been less encouraging (see below). According to animal experiments, oral and intranasal administration of antigen can also lead to induction of cytotoxic T-cell immunity (53–55) that may counteract its tolerance-promoting effects. This might need more attention in current trials where autoantigens are administered *via* mucosal routes. In animal models, a promising new strategy to induce regulatory immunity and protection from autoimmune diseases including diabetes is genetic (that is, DNA) vaccination with plasmids that encode an autoantigen (56). This strategy will be discussed later in this review.

Current clinical trials based on strategies developed in NOD mice

Strategies employed currently in clinical trials to prevent T1DM include the use of autoantigens in a tolerance-promoting form, targeting T-lymphocytes with antibody, protecting islet β-cells from the action of inflammatory cytokines and non-specific stimulation of regulatory cell types. Most of the ongoing trials, which are summarized in Table 2, are based on strategies developed in the NOD mouse. We will describe these trials shortly.

Anti-CD3 monoclonal antibody

hOKT3γ1[Ala-Ala] is a humanized non-activating anti-CD3 antibody engineered to lack Fc-Receptor binding domains (57). Preclinical studies in the NOD mouse showed that antibody treatment could effec-

Table 1. Examples of protocols that prevent diabetes in NOD mice and have a relevant target mechanism.

Protocol	Mechanism of action	Antigen specificity	Reference
Treatment with an antibody against:			
CD3	Depletion or inactivation of islet-infiltrating T cells/induction of regulatory immunity	no	(23, 24)
CD4/CD8	Depletion/inactivation of CD4 or CD8 T cells	no	(25)
TCR	Depletion/inactivation of T cells	no	(26)
CD86(B7.2)	Interference with T cell activation	no	(27)
CD45RB	Interference with T cell activation	no	(28)
CD40L	Interference with T cell activation	no	(29)
VLA-4 (α4-integrin)	Prevention of lymphocyte accumulation in islets and/or activation of diabetogenic T cells	no	(30, 31)
MAdCAM-1	Prevention of lymphocyte accumulation in islets and/or activation of diabetogenic T cells	no	(32)
ICAM-1/LFA-1	Prevention of lymphocyte accumulation in islets and/or activation of diabetogenic T cells	no	(33)
Treatment with:			
adjuvant (IFA)	immune deviation	no	(34)
vitamin D3 deriv.	immune deviation	no	(35)
αGal-ceramide	activation of regulatory NK T cells	no	(36)
interleukin 4	immune deviation/regulatory cells	no	(37)
interleukin-10	immune deviation/regulatory cells	no	(38, 39)
nicotinamide	protection of target β-cells against NO	no	(40)
subcutaneous insulin	Inactivation of pathogenic T cells	yes	(41, 42)
oral, intranasal or aerosol insulin	Induction of regulatory T cells/inactivation of pathogenic T cells	yes	(43–46)
insulin-encoding DNA-plasmid	Induction of regulatory T cells/inactivation of pathogenic T cells	yes	(47)
intranasal GAD peptides	Induction of regulatory T cells/inactivation of pathogenic T cells	yes	(48)
GAD-encoding DNA-plasmid	Induction of regulatory T cells/inactivation of pathogenic T cells	yes	(49, 50)
intrathymic GAD	Deletion of GAD-specific T cells from the pool of circulating mature T cells	yes	(51)
HSP 60	Inactivation of pathogenic T cells	yes	(52)
p277 of HSP 60	Inactivation of pathogenic T cells	yes	(52)

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Table 2. Currently ongoing clinical trials to prevent diabetes.

Trial	Principle/hypothesis	Evidence from NOD mice	Status	Results[a]
hOKT-3γ1	Inactivation of T cells. Induction of regulatory immunity	yes	Phase I-II	+
p277 of HSP-60	Induction of tolerance towards one autoantigen	yes	Phase I	+
insulin i.v. and s.c.[b]	Induction of tolerance towards one autoantigen	yes	Phase II	–
insulin s.c. in adjuvant (IFA)[c]	Induction of tolerance towards one autoantigen	yes	Phase II	N.A.
insulin *via* oral route	Induction of tolerance towards one autoantigen	yes	Phase II	N.A.
insulin *via* intranasal route	Induction of tolerance towards one autoantigen	yes	Phase III	N.A.
GAD 65 s.c.	Induction of tolerance towards one autoantigen	yes	Phase II	N.A.
nicotinamide *via* oral route	Protection of islet β-cells	yes	Phase III	–
IFN-α	Modulation of immune response	yes	Phase II	N.A.
Lactobacillus *via* oral route	Stimulation of regulatory NK-T cells	yes	Phase II	N.A.
Apl[d] of insulin B-chain	Induction of tolerance to insulin	yes	Phase II	N.A.
Vitamin D3 derivative	Modulation of immune response	yes	Phase II	N.A.
Exclusion of bovine proteins from diet in infancy	Avoidance of early immune response towards putative 'mimics' of autoantigens and/or sensitization of immature gut to foreign proteins	not directly	Phase III	N.A.

[a] Results: + positive, – negative results; N.A. results not available yet; [b] i.v. = intravenous administration; s.c. subcutaneous administration; [c] IFA = incomplete Freund's adjuvant; [d] *Apl* = altered peptide ligand.

tively 'cure' diabetic mice, restoring normoglycemia (24) or prevent disease development in pre-diabetic mice (58, 59). The antibody appears to bind to all CD3 expressing T-cells resulting in partial T cell receptor (TCR) signalling, which then has different outcomes depending on the cell type triggered. The overall outcome being killing or anergy induction in Th1 type cells (producing interleukin-2 or interferon-γ) and stimulation of Th2 type cells (cells producing interleukin-4 or interleukin-10) (60, 61). In an intervention trial, a 14 day course of intravenous hOKT3-γ1[Ala-Ala]) was used in recent onset (within 6 weeks) T1DM patients and followed for one year. Treatment resulted in sustained or improved C-peptide responses in 9 out of 12 treated patients compared with a sustained response in only 2 out of 12 control patients. Treated patients also needed significantly less insulin over the year following diagnosis. A transient depletion (36%) of lymphocytes followed antibody treatment, which later returned to normal levels. Responding patients had a decreased ratio of CD4:CD8 T cells after repopulation. Mild side effects included anti-idiotype antibodies, mild fever and an eczematous dermatitis-like rash. This trial is now being expanded to a multicentre phase II trial involving about 80 patients after these promising results. Further details are available at http://www.immunetolerance.org/research/autoimmune/trials/herold1.html

Hsp-60 peptide p277

One of the many self antigens, which are reacted against in T1DM, is heat shock protein 60. NOD mice contain autoreactive T cells specific for peptide 277 derived from this protein and immunisation of adult NOD mice with this peptide could prevent and occasionally revert diabetes (52). The mechanism of action is believed to be stimulation of Th2 type hsp60-reactive T cells resulting in a change in the cytokine milieu away from inflammatory cytokines (62). DiaPep277 is the human form of this peptide, which has been modified at two residues to increase stability *in vivo* (63). In a randomised double blind phase II trial DiaPep277 was injected subcutaneously in mannitol and vegetable oil at 0, 1 and 6 months following entry into the trial (63). Adult male patients were on average 12–15 weeks post-diagnosis and were followed for only 10 months. At the end of the study mean C-peptide levels had been maintained in treated patients whereas they had fallen in controls. Insulin requirements were also significantly lower at 10 months. Further phase II and phase III trials are now beginning based on these suggestive results.

Subcutaneous insulin therapy

Insulin has long been viewed as a primary target for tolerisation therapy in T1DM. It was shown that incidence of diabetes was significantly reduced in NOD mice given a low dose of prophylactic insulin from weaning until 180 days of age (41). Similar findings were also observed in the BioBreed rat, another animal model of T1DM (64). This phenomenon was believed to be due to 'beta-cell rest' in which there was a lower requirement for insulin secretion by the beta cells and less release of islet autoantigens associated with insulin secretion. These findings paved the way to the establishment of the Diabetes prevention trial (DPT-1) in which either subcutaneous or oral insulin was given to high risk relatives of T1DM patients. In the subcutaneous branch of the trial 84,228 relatives were assessed immunologically, metabolically and genetically for risk of disease

development over 5 years (DPT-T1D study group 2002). A group of 169 high risk (>50% over 5 years) relatives then underwent intervention of daily s.c. insulin injections and 170 controls received no treatment. The incidence of diabetes after 5 years was identical in both groups (69 *versus* 70 diabetic subjects). The reason for the negative outcome in the DPT-1 trial compared with the positive results in animal models and a pilot trial in humans (65) is unknown. It may be due to the dosage protocol or the difference in time of intervention. In mice, therapy began at a young age before the development of insulitis whereas relatives were selected on the basis of showing signs of autoimmunity (presence of islet autoantibodies) already.

Mucosal insulin

The second part of the DPT-1 trial is ongoing and used the 'intermediate' risk (25%–50% risk of developing T1DM over 5 years) relatives of T1DM patients. Patients receive continuous oral insulin or placebo and are being followed for 6 years for diabetes development. This intervention was not predicted to prevent diabetes by 'beta-cell rest' as subcutaneous insulin was but rather through active tolerisation upon antigen uptake at a mucosal surface (66). In NOD mice, an active (transferable), form of tolerance can be induced by feeding mice insulin but it is highly dose-dependent (43, 44). Therefore it is likely that in transferring this therapy to the human situation the dosage protocol will be critical.

A second major trial, the diabetes prediction and prevention project (DIPP), also seeks to tolerise individuals predicted to have a high risk of progression to T1DM by mucosal insulin administration. In this study babies are screened at birth for genetic HLA markers predisposing them to T1DM and then undergo immunological follow up. At risk children are then enrolled in a prevention trial to test intranasal insulin *versus* placebo for its ability to prevent or delay disease onset over three years. Aerosol insulin and intranasal insulin peptide administration have both been shown to be efficacious in prevention of diabetes in the NOD model (45, 46), although intranasal antigen administration has also been shown to induce potent cytotoxic T-cell immunity (67). For further details, see http://www.utu.fi/research/dipp/engdexx.htm

Subcutaneous insulin B chain in IFA

Another trial also attempts to induce tolerance to the metabolically inactive B chain of insulin by subcutaneous injection in Incomplete Freunds Adjuvant (IFA). In the NOD mouse, subcutaneous insulin or insulin B-chain given in IFA generated a transferable suppressive effect (42). In this study patients with insulin autoantibodies receive one injection within one month of diagnosis of T1DM and will be followed for two years. For further details, see http://www.immunetolerance.org/research/autoimmune/trials/orban1.html

Subcutaneous GAD65 in alum

An important early autoantigen in diabetes in both the NOD mouse and humans is GAD65 (51). A phase II trial will test the ability of recombinant GAD65 in alum to halt disease progression in recent onset patients. The vaccine called Diamyd will be injected subcutaneously two times four weeks apart in a range of doses. For further details, see http://www.diamyd.com/docs/research.html

Nicotinamide

A different approach, which is not antigen specific, attempts to use high doses of the B-vitamin nicotinamide to prevent or delay T1DM onset. Nicotinamide acts on the islet beta cells themselves making them more resistant to autoimmune attack. Nicotinamide is thought to primarily target the enzyme poly(ADP-ribose)polymerase (PARP) which is upregulated early after exposure to nitric oxide or reactive oxygen intermediates. Such exposure causes depletion of intracellular nicotinamide adenine dinucleotide (NAD^+) and results in death of sensitive cell types such as beta cells (68). Early nicotinamide treatment of NOD mice prevented diabetes partially and reduced insulitis severity (40). The results of initial clinical trials (69–71) have been controversial, since, while Chase et al. reported no effect of oral administration of nicotinamide, both Mendola and collaborators and Pozzilli et al. observed an increased stimulated C-peptide secretion in postpubertal patients on nicotinamide.

Nicotinamide has also been used in prevention trials with relatives of T1DM patients. One German study of 25 nicotinamide treated and 30 placebo control subjects showed no effect on disease progression (72). However, a larger study in New Zealand involving schoolchildren who had islet-cell autoantibodes obtained results that suggested a protective effect (73). The European nicotinamide diabetes intervention trial (ENDIT) is a larger clinical trial involving 552 high-risk relatives taking daily oral nicotinamide or placebo. For further details and for the recently disclosed negative results of this trial, see http://www.bris.ac.uk/Depts/DivMed/endit.html

Interferon-α

One successful strategy to prevent disease in the NOD

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mouse involves altering the cytokine milieu to an anti-inflammatory, i.e., interleukin (IL)-4 and IL-10 containing environment. This has been done by injection of these cytokines, transgenic expression or by stimulation of cells which can produce them endogenously. Brod and co-workers have fed NOD mice with interferon-α, which suppressed diabetes and stimulated mitogen induced IL-4, IL-10 and interferon (IFN)-γ production in splenocytes (74). This was then trialled in 10 newly diagnosed T1DM patients resulting in preserved beta-cell function in 8 of these patients after 12 months (75). This study has now been expanded to a larger phase II trial involving 120 newly diagnosed patients taking oral IFN-α daily. It is unclear however what the exact mechanism of this therapy is, as interferon administered orally does not appear to be adsorbed into the bloodstream (76). Additionally, the presence of transgenically expressed IFN-α in the islet beta cells themselves actually precipitates diabetes (77). As such it is possible that interferon-α acts on lymphocytes in the gut, which then circulate to have a wider regulatory effect.

Oral administration of lactobacillus

Another study also attempts to modulate the immune response *via* the gut. Maclaren and co-workers are conducting a trial of oral lactobacillus in recently diagnosed T1DM patients. They propose that this will stimulate natural killer cell-like T (NKT) cell activity as administration of lactobacillus plantarum to children with HIV boosted this cell-subset (78). NKT cells are a regulatory-cell subset which have been reported to be deficient in T1DM patients (79, 80) although a more recent study utilising a more direct tetramer based method of identifying NKT cells refutes this claim (81). In the NOD mouse both transfer of this cell subset or injection of a glycolipid ligand to activate existing NKT cells can prevent diabetes (36, 82, 83). Lactobacillus casei feeding to NOD mice prevents disease (84) and various lactobacilli strains have been shown to be strong stimulators of IL-12 and IL-10 (85). However, strong evidence of lactobacillus stimulation of NKT cells has yet to be demonstrated.

Altered peptide ligand NBI-6024

An interesting antigen specific therapy involves the identification of peptides with similar sequences to immunodominant epitopes, which have modulatory activity. In the NOD mouse peptide 9-23 of the insulin B chain is recognised by a large proportion of pathogenic CD4 T-cells derived from the insulitic infiltrate (86–88). Alleva and colleagues searched for peptide analogues of $B_{(9-23)}$ which could inhibit $B_{(9-23)}$ specific T-cell responses and used one of these, NBI-6024, to test its therapeutic effect in the NOD mouse (89). Therapeutic altered peptide ligands are thought to function as competitive inhibitors of the native peptide by having a high binding affinity for MHC while concurrently engaging the TCR in a non-productive manner (90). They have also been shown to be effective in stimulating regulatory cell subsets, which can transfer protection between animals (91). The altered peptide ligand NBI-6024 is now being used in a large phase II study in which new onset T1DM patients will receive monthly injections of this medication at three different doses for two years.

1,25-Dihydroxy-Vitamin D3

The activated form of vitamin D3, 1,25-Dihydroxy-Vitamin D3, has been shown to act directly on the immune system *via* specific receptors on APCs and activated T cells (92). Its effects are immunosuppressive and include inhibition of IL-2 and IL-12 production and Th1-type responses (93). Importantly 1,25-Dihydroxy-Vitamin D3 has been shown to inhibit dendritic cell maturation, both *in vitro* and *in vivo* (94, 95). Administration of an analogue of 1,25-Dihydroxy-Vitamin D3 to NOD mice significantly reduced the incidence of diabetes (96) and enhanced the number of $CD4^+$ $CD25^+$ cells of regulatory phenotype in the pancreatic lymph node (97). 1,25-Dihydroxy-Vitamin D3 administered daily for 9 months in recently diagnosed T1DM patents is being trialled in a German study. For further details on this trial see http://www.roche.com/pages/downloads/science/pdf/rtdcmannh02-3.pdf

Withdrawal of cow's milk proteins from diet during infancy

According to one hypothesis (not directly derived from findings in NOD mice), exposure of the immature digestive tract in infancy to casein or other proteins in cow's milk formula or soy-based formula has a role in the early pathogenesis of type 1 diabetes. A casein-free diet has been tested in the NOD mouse after weaning and was shown to be effective in preventing diabetes (98). Although contradictory results were achieved from previous studies in humans evaluating whether introduction of formula during infancy is associated with the development of type 1 diabetes later in life (99, 100), a larger phase III clinical trial called TRIGR (Trial to Reduce IDDM in the Genetically at Risk) has been initiated to test if the hypothesis holds true or not. Infants that are determined to have a high risk of developing type 1 diabetes are eligible. After weaning from breast-feeding, they will receive hydrolyzed formula that does not contain intact proteins, or standard cow's milk-based formula. Infants will have at least a two-

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month exposure to the study formulae and then will be monitored for up to ten years. For further details, see http://www.trigr.org/about.html

Transgenic mouse models in the study of T cell-mediated autoimmunity against pancreatic islets

Investigations in humans with T1DM and studies performed in the NOD mouse model have clearly demonstrated the crucial role of autoreactive CD4 helper and CD8 effector T cells in the pathogenesis of autoimmune diabetes (101). In NOD mice, numerous autoantigens are recognized by these T cells (see above). The relevance of these antigens for disease initiation and progression, and the mechanisms of their processing and presentation, however, are still unclear. Consequently, techniques and tools other than the NOD model had to be developed. The most valuable models were generated by genetically introducing well characterized model antigens into islet-β-cells, under the assumption that such neoantigens will be handled like endogenous islet antigens by the immune system (102). Transgenic neoantigens, however, are often aberrantly expressed in the thymus, resulting in the deletion of endogenous transgene-specific T cells. This problem could be overcome by intravenous injection of specific T cells, which are usually obtained from congenic T cell receptor – transgenic mice. Since their antigen (the model autoantigen) is not expressed in the donor mice these T cells are not negatively selected in the thymus. Importantly, the absence of antigen during T cell development ensures a completely naïve phenotype of the autoreactive T cells. This is crucial because it allowed, for the first time, investigation of the activation of T cells specific for a pancreatic self antigen *in vivo* (103). The other major advantage of adoptive transfer experiments is the ability to label the T cells with fluorescent dyes prior to injection, which allows them to be easily tracked *in vivo*. This technique can also reveal their activation and proliferation through analysis of cell division (104, 105). These innovations allowed many important aspects of T cell-mediated autoimmune responses to be addressed, including the site of activation of the autoreactive T cells, the influence of their numbers, their fate and the type of antigen-presenting cell responsible. Although parameters such as T-cell affinity and epitope spreading and recruitment of other autoreactive T cell clones are not taken into account, this approach has added considerably to our knowledge of immune-mediated destruction of islet β-cells by allowing us to dissect the actions of relevant components and their interactions which finally result in disease (102).

The approach described above has been particularly successful in elucidating the role and physiology of CD8 T effector cells. As compared to CD4 T helper cells, CD8 T cells are difficult to maintain *in vitro* as T cell lines, mainly because of their cytotoxicity. They quickly destroy APCs *in vitro*, and thus deprive themselves of antigenic survival signals. Furthermore, the MHC class I molecules, which are recognized by CD8 T cells, are expressed by nearly all murine cells, including T cells, so that CD8 T cells could potentially kill each other *in vitro*. These problems were overcome by generating naïve, and therefore unarmed CD8 T cells in transgenic donor mice. Such T cells became cytotoxic only after transfer into recipient mice expressing antigen. Transgenic CD8 T cells specific for certain MHC class I molecules were first generated (106). These T cells were introduced into transgenic mice expressing MHC I molecules under the influence of the rat insulin promoter (RIP) in pancreatic islet cells. This resulted in immune tolerance, unless the T cells were supplied with inflammatory mediators (107). These experiments demonstrated that autoreactive CD8 T cells can in principle destroy β-cells and cause diabetes (102) although their specificity for antigenic peptide was unclear. Therefore, they did not allow investigation of the processing of islet antigens. This limitation was overcome in the next generation of CD8 T cell transgenic systems. The first of these models, the RIP-mOVA model, expressed ovalbumin (OVA) as a model antigen in pancreatic islet cells. OVA-derived peptides could be detected with transgenic OVA-specific CD8 and CD4 T cells derived from OT-I and OT-II mice, respectively (OT-I and OT-II refer to the transgenic OVA-specific T cells, called OT-I and OT-II cells, that are produced by these transgenic mice). These experimental systems demonstrated clearly that both naïve CD4 and CD8 T cells with specificity for a pancreatic self antigen are activated by dendritic cells in the pancreatic lymph nodes, and not in the islets (108) (Fig 1). The DCs presumably took up β-cell antigens in the islets and carried them to the pancreatic lymph node for presentation to naïve T cells. In the case of CD8 T cells, these results demonstrated so-called cross-presentation, which denotes the presentation of extracellular antigens with MHC I molecules to CD8 T cells.

Since then, numerous observations of cross-presentation and cross-priming *in vivo* have been described, not only of transgenic self-antigens in models like the RIP-mOVA or the hemagglutinin system (109), but also of tumour (110) and viral antigens (111, 112). Thus, the basic mechanisms of antigen presentation and T-cell activation *in vivo* that were uncovered in transgenic systems have been verified for many 'natural' antigens. This is not surprising, because indirect presentation of antigen

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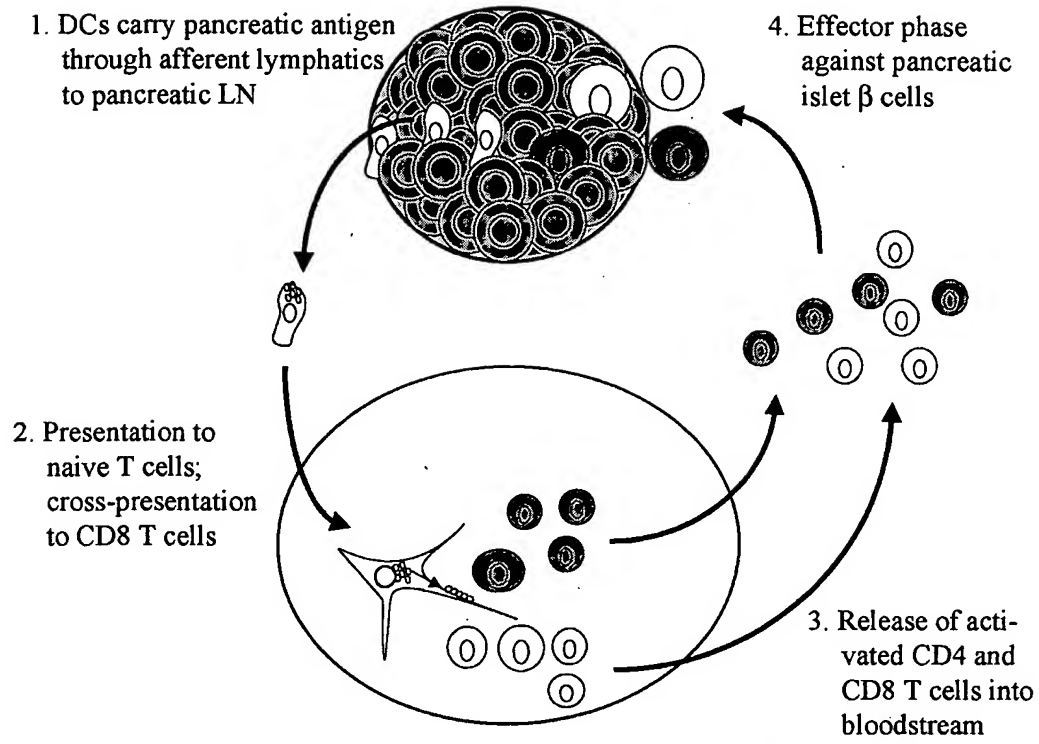

Figure 1. Antigen capture by dendritic cells in islets and their migration to the pancreatic lymph node for presentation of β-cell antigens to T lymphocytes. Antigen-presentation *via* both MHC II and MHC I pathways enables both CD4 and CD8 T cells to be activated. Activated T cells can enter non-lymphoid tissues such as pancreatic islets in search for antigen-expressing target cells (see text for details).

to T cells – both to CD4 and/or CD8 T cells – offers advantages to the immune system in the battle against infections antigens. Only indirect antigen presentation (in the case of CD8 T cells, cross-presentation) allows an immune response to be mounted against viruses that functionally compromise, or simply avoid antigen-presenting cells (103).

These basic mechanisms of antigen presentation, which were uncovered in model systems such as the RIP-mOVA system, were found to be important also for the pathogenesis of diabetes in NOD mice, in which CD8 T cells have emerged as important mediators of disease (113–117). TCR-transgenic NOD mice have been generated, and in many of these, the CD8 T cells destroyed pancreatic islets, whereas the CD4 T cells played more or less relevant helper roles (115, 118, 119). In NOD mice, autoreactive CD4 T cells appear to play an important role in diabetogenesis, and also their activation occurs in the pancreatic lymph nodes (120). Interestingly, presentation of autoantigen to autoreactive CD4 and CD8 T cells is age-dependent. It did not occur in very young animals (120), suggesting that developmental changes in the pancreatic tissue cause the release of autoantigens to the pancreatic lymph node. In this respect, a wave of physiological apoptotic β-cell death, which occurs in rodents at 14–17 days after birth (121), and in humans at birth (122), may be relevant. During such a wave, autoantigens are released from apoptotic islet cells and may activate autoreactive T cells with specificity for β-cell antigens (101, 123). Antigens released from apoptotic cells are taken up particularly well by dendritic cells (124–126) and normally, induce T cell tolerance (127). Why antigen-presentation results in autoimmunity in some individuals is not known, but inflammatory signals caused by accompanying infections (128), differences in the T cell repertoire, genetic susceptibility differences and dysregulated cellular death may be involved. Also, unphysiological β-cell death might cause the release of islet antigens in an immunogenic setting, and induce autoimmunity rather than tolerance (101). Tissue remodelling and replacement of β-cells occur also later. In the RIP-mOVA system, pancreatic tissue antigens were observed to be constantly shuttled to the draining lymph nodes of adult animals, where they were presented to autoreactive T cells (108, 120). These T cells indeed caused diabetes, but only when their precursor frequency was unphysiologically high (103). Physiological numbers of T cells were tolerized

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by deletion before they could destroy all β-cells. Thus, cross-presentation of pancreatic self-antigen led to deletional CD8 T-cell tolerance, which was termed cross-tolerance (129). Cross-tolerance has also been shown in other transgenic systems, and its mechanisms have been elucidated (109). However, in some systems, most notably in those examining antigens derived from the lymphochoriomeningitis virus (LCMV), specific CD8 T cells ignored this self antigen (130) but were not deleted. One possible explanation is that various antigens are handled differently by the immune system. In this case, however, pathogens might easily evolve strategies to escape immune surveillance. An alternative explanation came from studies investigating the influence of antigen dose on cross-presentation. Only high antigen levels were cross-presented and induced cross-tolerance (131–133) while low dose self antigens were ignored, in which case the immune system must rely on ignorance to avoid autoimmunity. Thus, the level of antigen expression appears to determine the mechanism by which CD8 T-cell mediated autoimmunity is avoided.

Ignorance of self antigen allows autoreactive T cells to survive within the T cell repertoire. Such T cells could theoretically unleash their destructive potential if they were activated by other means, for example by pathogens that are similar in antigenic structure. This sword of Damocles has first been demonstrated in the LCMV system, where mice expressing determinants of the LCMV virus in pancreatic islets became diabetic after infection with the virus (134–135). Such a mechanism may explain why immune diseases are often observed after viral infections. Direct evidence for antigenic mimicry as a mechanism of diabetes induction, however, is scarce (136). In contrast to ignorance or to the induction of anergy or TCR downregulation in autoreactive T cells (137, 138) deletion of autoreactive T cells removes the threat of autoimmunity permanently by eliminating potentially harmful effectors. However, this mechanism can also fail, for example if the precursor frequency of the autoreactive CD8 T cells is too high, or when autoreactive CD4 T cells are present (103). Transgenic CD4 T cells specific for islet self-antigens appear to be less efficient as direct mediators of diabetes than CD8 T cells (139, 140). However, they act by delaying the deletion of CD8 effector T cells (139), by mediating their entrance into pancreatic islets (115) or by supporting their effector phase in the islets (107). But also the CD4 T cells themselves are subject to peripheral tolerance. CD4 T cells can be deleted (141) or functionally silenced (137). Also Th1 → Th2 diversion may happen (142) and regulatory $CD25^+$ CD4 T cells, that actively suppress immune responses (143) can be generated.

Molecular mechanisms regulating immune response to islet-antigens

As described above, antigens derived from islet-β-cells are under continuous surveillance by the immune system. Whether this antigen-presentation leads to expansion of islet-specific T lymphocytes and to the development of anti-islet immunity (Fig 1) – or to their silencing – is of crucial importance. Molecular mechanisms underlying this distinction are gradually becoming unravelled. Signalling via cytokine- and costimulatory receptors is of particular importance (144–147). For example, expression of the costimulatory ligand B7.1 or TNF-α on islets, and signalling between CD40L and CD40 can lead to the breakdown of the tolerant state to islet-antigens, as has been shown in NOD mice by antibody treatments and by expression of TNF-α or B7.1 on β-cells (148–150). The action of TNF-α depends, however, on the timing of its action and can also suppress β-cell destruction (149, 150).

The TNF- and TNF-receptor families include several members that may still turn out to be important in the regulation of islet-immunity. A good example is the demonstration that signalling through the TRANCE-RANK receptor-ligand pair is involved in the generation of CD25 + CD4+ regulatory T cells in the pancreatic lymph node (151). These cells were shown to be able to restrain diabetogenic CD8 effector T cells. The existence of regulatory T cells seems to be controlled by costimulatory molecules, because in the NOD mouse, lack of B7-2 or CD28 leads to acceleration of diabetes *via* impaired action of $CD25^+$ CD4 regulatory T cells (152).

Functional silencing, that is 'anergy', is traditionally considered to result from TCR-ligation without simultaneous costimulation but may in fact require costimulation. Accordingly, CTLA-4 is an important negative regulator of the activity of T cells (153), and abrogation of its function leads to acceleration of diabetes in BDC2.5 NOD mice (153). Also regulatory cells including $CD25^+$ CD4 cells (143) and Treg1 cells (154), are partly anergic themselves. Cytokines IL-10 and transforming growth factor (TGF) -β, produced by regulatory T cells, are important in peripheral tolerance via their inhibitory actions on dendritic cell activity and on T cell proliferation and differentiation (155, 156). Which role these regulatory cells play in human disease is not yet clear but is under intensive research.

Receptors that recognize pathogen-associated molecular patterns (PAMP) and are thus called pattern recognition receptors (PRR) are also important in the generation of immune responses (157). These receptors are expressed on cells of the innate immune system including dendritic cells and couple with intracellular signal transduction pathways that reg-

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ulate gene expression. Binding of a ligand (i.e., a foreign pathogen) to such receptors can thus upregulate various functions of dendritic cells. Although it is quite unclear if PRR have any role in the pathophysiology of islet-specific autoimmunity, these receptors and their signalling pathways deserve attention when attempting to manipulate antigen presentation in pancreatic lymph node.

Consequently, costimulatory and/or death-receptors and cytokines expressed by the cells of our immune system form an intricate network of interacting receptors and ligands that influence the fate of islet-reactive T lymphocytes. This network contains a multitude of potential drug targets for attempts to restore tolerance to peripheral tissue antigens such as islet-antigens, some of which are depicted in Figure 2.

Selective modulation of immune response to islet-antigens

Restoration of tolerance to islet β-cells without compromizing general immune function requires that immunomodulation selectively targets the immune response to β-cells. So far, tolerance has been induced experimentally by introducing the antigen in a controlled fashion, i.e., without adjuvants, as a soluble protein and preferably *via* mucosal surfaces or the intravenous route (158, 159). As discussed above, the prospects of these manipulations as immune therapy to diabetes, however, remain very uncertain. Tolerance can also be induced in T cells specific for any given antigen by complexing antigenic peptides with MHC molecules to produce 'custom-made' T cell receptor ligands that are administered without adjuvants and thus in the absence of costimulatory signals (160, 161). This, however, requires detailed knowledge of the immunodominant epitopes of the antigen, and when effective, is likely to be strictly restricted to a narrow specificity of T cells that may not alone be responsible for disease pathogenesis. Thus, multiple T cell receptor ligands would need to be administered unless some of them would be able to induce active regulatory immunity (161).

Current knowledge of the cellular and molecular mechanisms underlying regulation of peripheral tolerance to islet-antigens suggests that new strategies of therapeutic manipulation of the detrimental islet-specific immune response await to be discovered. This would, however, require novel ways of introducing islet-antigens to the immune system in a way that would allow a tolerance-regulating mechanism to be

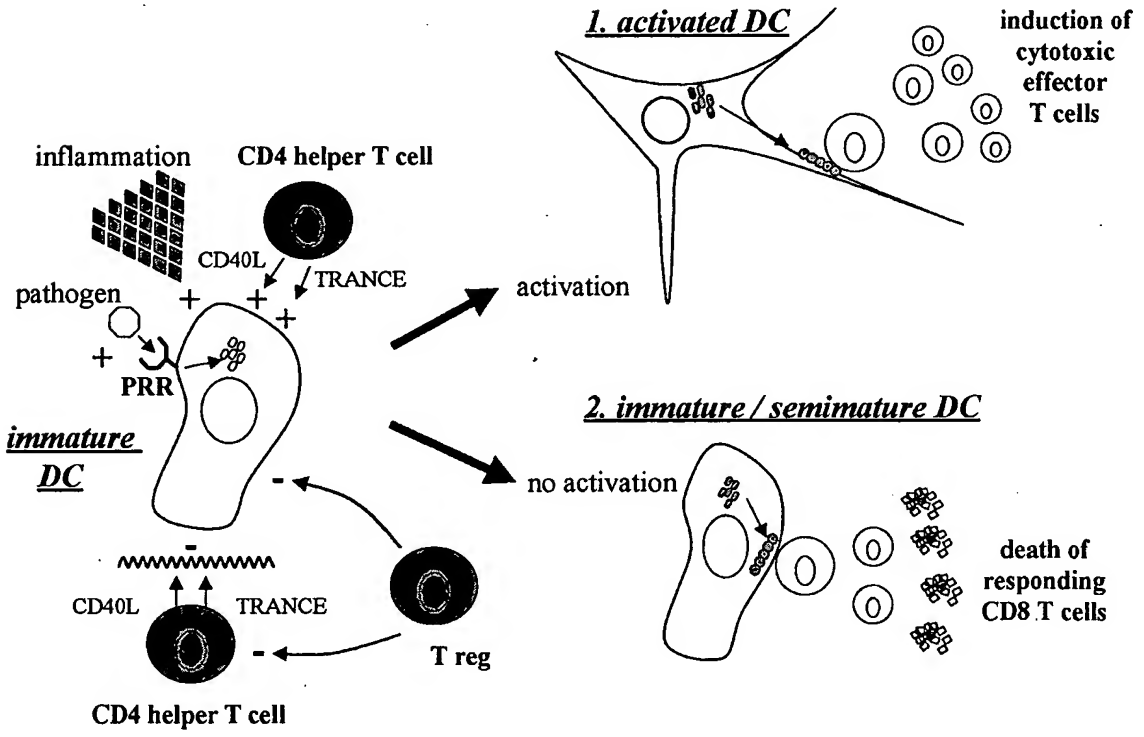

Figure 2. Factors that promote the activation of immature dendritic cell (DC) to become a potent antigen-presenting cell (1. activated DC) after endocytosis of antigen; and the role of regulatory T cells (Treg, e.g., CD25 + CD4 T cells) as opponents of the activation of immature DC (2. immature/semimature DC). PRR = pattern recognition receptor.

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introduced simultaneously with the antigen in a strictly localized manner. Because T cells recognize antigen only when appropriately presented to them, this is possible only during T-cell interaction with an antigen-presenting cell or a target cell. In type 1 diabetes, the target cells (i.e., islet-β cells) are obviously beyond selective manipulation unless xeno-transplantation of pig islets derived from transgenic pigs (whose islets would be made to express immuno-modulatory agents) is considered relevant as a treatment option. Interaction with an APC which is presenting an autoantigen is the other antigen-specific interaction that a diabetogenic T cell commits itself to. Therefore, immunomodulation should probably be targeted here. If this APC cell were made to express an immunomodulatory agent, it could perhaps selectively target this agent to the T cell that is engaged with it *via* its antigen-specific receptor (162). This might re-direct the T cell to a less aggressive behaviour instead of its expansion and maturation into a clone of islet-destructive T cells. The immunomodulatory agent could be a death-inducing ligand such as FasL, TRAIL, TNF or PD-L1 (163–165). Experimental evidence suggests that expression of FasL on dendritic cells renders them tolerogenic (166, 167) although this may not always happen (168). In fact, we have observed that ligation of Fas during T cell contact with dendritic cells costimulates a fraction of the responding T cells (169) which could complicate its effects as a death-ligand. For other death-receptor ligands, data do not yet exist.

Alternatively, the immunomodulatory agent could be a cytokine such as IL-10 or TGF-β, because these cytokines regulate the costimulatory activity of the antigen-presenting cell and proliferation and polarity of the responding T cell as discussed above (155, 156). For therapeutic purposes, this type of targeted immunomodulation could be achieved by *in vitro*-treatment of autologous antigen-presenting cells with the immunomodulatory agent together with the antigen before re-injection, or by genetic modification to make these cells express the immunomodulatory agent themselves. Also, although B lymphocytes as well as monocytes can present antigen to T cells, dendritic cells would likely suit best to be used as modified antigen-presenting cells. This is because only dendritic cells are able to induce a response both in naïve and antigen-experienced T cells (170), and because B lymphocytes have an intrinsic property of directing islet-reactive T cells into diabetogenic behaviour at least in the NOD mouse (171, 172).

Modification of antigen-presentation from dendritic cells – a therapeutic option?

Dendritic cells can be propagated from peripheral blood of humans with the aid of cytokines granulocyte macrophage colony stimulating factor (GM-CSF) and IL-4 (173, 174). Such dendritic cells could be treated *in vitro* with an immunomodulatory agent or perhaps transfected with a gene construct to make them express the immunomodulatory agent themselves and pulsed with antigen before re-injection back into the same individual. *In vitro* cultured and antigen-pulsed dendritic cells from healthy individuals can, in fact, either induce a potent immune response or tolerance depending on their prior *in vitro*-treatment when re-injected (175, 176). The limitations of this approach would come from the amount of work and potential safety risks introduced by *in vitro* propagation of autologous cells. These limitations would not apply to the use of purified DNA as a 'vaccine' (177). The discovery that injecting 'naked' DNA can induce immunity was made a decade ago (178), and vaccination with 'naked' DNA represents a promising strategy for inducing cell-mediated immune responses (including cytotoxic CD8 T cells) (177, 178). Purified plasmid DNA encoding both an autoantigen and an immunomodulatory agent could therefore work as a 'vaccine' that might elicit a modified immune response resulting in tolerance instead of effective cell-mediated immunity. Unlike cells that need to be autologous, a vaccine consisting of recombinant DNA once validated could potentially be applied to individuals of a diverse genetic background (i.e., irrespective of MHC haplotypes or other disparate features). Thus, a 'vaccine' for people at risk of developing T1DM could perhaps become available on a large scale. The safety risks of introducing foreign recombinant DNA would probably be small if vectors that are able to incorporate into host genome (i.e., retroviral vectors) would not be applied.

In the NOD mouse, a few studies already exist in which DNA-plasmids encoding insulin, its B chain, GAD or hsp 60 either alone or together with a plasmid encoding a regulatory cytokine (e.g., IL-4) have been injected as a 'vaccine' to induce tolerance (47, 179–181). DNA injected as a 'vaccine' is taken up by cells in the tissue including dendritic cells which express the antigen and present it to T cells (182). In many cases this has resulted in regulatory immunity and protection from disease. This is noteworthy, given the capacity of DNA-vaccination to induce effective cytotoxic T-cell immunity in tumour and viral disease models (183, 184). Hence, the control of possible adverse immune reactions deserves careful attention when using this strategy. We believe that simultaneous expression of the autoantigen with an immunomodulatory agent could be a possible way to better control such adverse reactions and to target immunomodulation most effectively to pathogenic T cells. Intensive research is

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yet needed in models such as the RIPmOVA -model and the NOD mouse to identify the most effective immunomodulatory molecules and the most relevant islet-autoantigens that these should be combined with.

A promising strategy to induce tolerance towards a defined antigen is to make a fusion protein consisting of the antigen and an antibody to an endocytosis receptor expressed on immature dendritic cells. Accordingly, the model antigen ovalbumin when conjugated to an antibody against the DEC-205 receptor, induced antigen presentation in dendritic cells that remained in an immature state (185). Presentation of antigen *via* immature dendritic cells rendered mice tolerant to subsequent challenge with the same antigen showing that targeting the antigen thoughtfully to the immune system may elicit antigen-specific tolerance.

We have discussed here the role of animal models in development of therapeutic strategies to induce immune tolerance to islet-autoantigens and envisioned some novel approaches to modify antigen-presentation from dendritic cells to induce tolerance (Fig 3). These strategies and approaches are currently under investigation in many laboratories including our own laboratories. Via innovative and careful work in animal models of islet-autoimmunity some of these approaches may be transformed into strategies that could be applied as specific immunotherapy to type 1 diabetes. Compared to treatment with general immunomodulatory or immunosuppressive agents, or other general, although certainly effective, treatment options like bone marrow reconstitution (186), tolerance-promoting presentation of autoantigens to the immune system would offer a worthwhile choice.

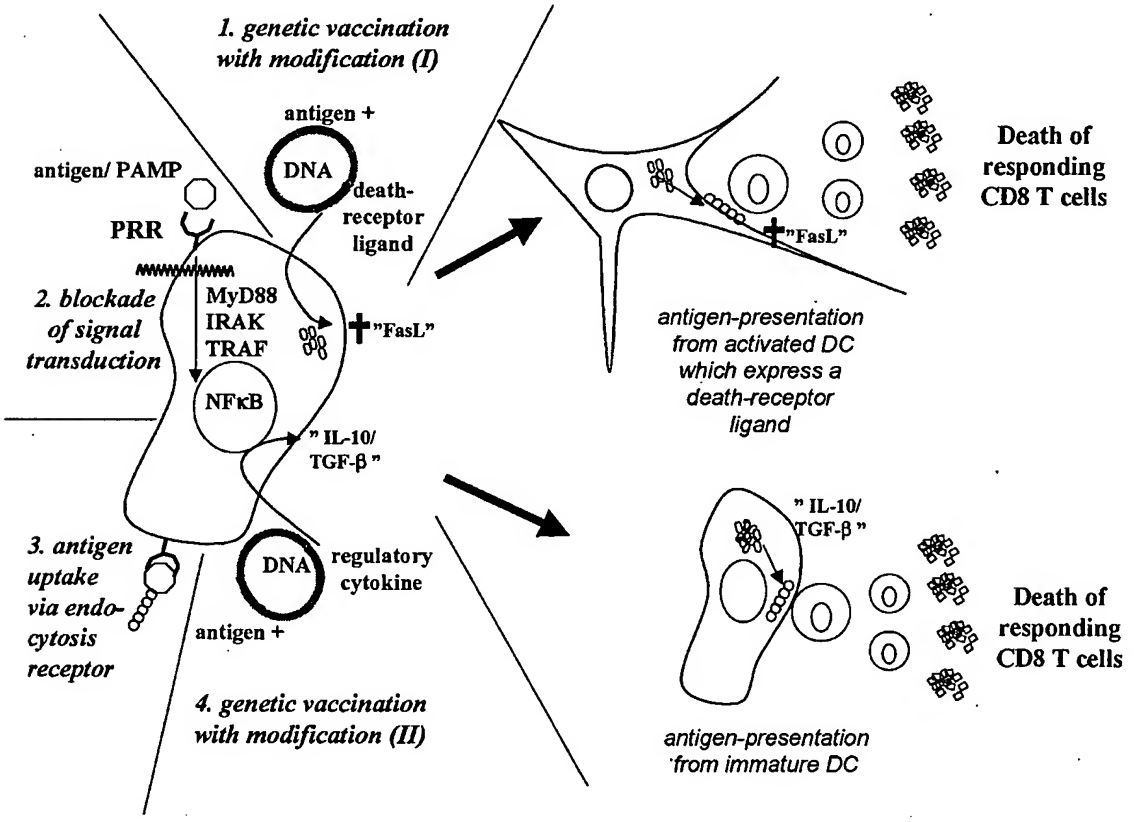

Figure 3. Four potential strategies for manipulating antigen-presentation in dendritic cells to induce tolerance. 1. Genetic (i.e. DNA) vaccination with a plasmid that encodes antigen and a death-receptor ligand (FasL, TRAIL, PD-L1/2); 2. Blockade of signal transduction from pathogen recognition receptor (PRR) by e.g., genetic modification of DC or antisense oligonucleotides; 3. Conjugation of antigen to a structure that binds to an endocytosis receptor (e.g., DEC-205) expressed on immature DC; 4. Genetic (i.e., DNA) vaccination with a plasmid that induces production of a regulatory cytokine (TGF-β, IL-10).

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References

1. Knip M. Natural course of preclinical type 1 diabetes. Horm Res 2002;57 Suppl 1:6–11.
2. Notkins AL. Immunologic and genetic factors in type 1 diabetes. J Biol Chem 2002;277:43545–8.
3. Roep BO. T-cell reactivity to beta-cell antigens in human insulin-dependent (type 1) diabetes mellitus. Implications for diagnosis and therapy. Clin Rev Allergy Immunol 2000;19: 265–75.
4. Bottazzo GF, Dean BM, McNally JM, MacKay EH, Swift PG, Gamble DR. In situ characterization of autoimmune phenomena and expression of HLA molecules in the pancreas in diabetic insulitis. N Engl J Med 1985;313:353–60.
5. Hanninen A, Jalkanen S, Salmi M, Toikkanen S, Nikolakaros G, Simell O. Macrophages, T cell receptor usage, and endothelial cell activation in the pancreas at the onset of insulin-dependent diabetes mellitus. J Clin Invest 1992;90: 1901–10.
6. Itoh N, Hanafusa T, Miyazaki A, Miyagawa J, Yamagata K, Yamamoto K, et al. Mononuclear cell infiltration and its relation to the expression of major histocompatibility complex antigens and adhesion molecules in pancreas biopsy specimens from newly diagnosed insulin-dependent diabetes mellitus patients. J Clin Invest 1993;92:2313–22.
7. Sutherland DE, Goetz FC, Sibley RK. Recurrence of disease in pancreas transplants. Diabetes 1989;38 Suppl 1:85–7.
8. Assan R, Bach JF, Czernichow P, Dupre J, Stiller CR, Eisenbarth GS, et al. Immunosuppressive drugs in diabetes. Lancet 1986;2:1097.
9. Gottlieb PA, Hayward AR. Cytokine and immunosuppressive therapies of type 1 diabetes mellitus. Endocrinol Metab Clin North Am 2002;31:477–95.
10. Akerblom HK, Knip M. Putative environmental factors in Type 1 diabetes. Diabetes Metab Rev 1998;14:31–67.
11. Knip M, Akerblom HK. Environmental factors in the pathogenesis of type 1 diabetes mellitus. Exp Clin Endocrinol Diabetes 1999;107 Suppl 3:S93–100.
12. Atkinson MA, Eisenbarth GS. Type 1 diabetes:new perspectives on disease pathogenesis and treatment. Lancet 2001;358: 221–9.
13. Atkinson MA, Maclaren NK. The pathogenesis of insulin-dependent diabetes mellitus. N Engl J Med 1994;331:1428–36.
14. Atkinson MA, Maclaren NK. Islet cell autoantigens in insulin-dependent diabetes. J Clin Invest 1993;92:1608–16.
15. Harrison LC. Islet cell antigens in insulin-dependent diabetes: Pandora's box revisited. Immunol Today 1992;13:348–52.
16. Leslie RD, Atkinson MA, Notkins AL. Autoantigens IA-2 and GAD in Type I (insulin-dependent) diabetes. Diabetologia 1999;42:3–14.
17. Atkinson MA, Leiter EH. The NOD mouse model of type 1 diabetes: as good as it gets? Nat Med 1999;5:601–4.
18. Mathis DJ, Benoist C, Williams VE, 2nd, Kanter M, McDevitt HO. Several mechanisms can account for defective E alpha gene expression in different mouse haplotypes. Proc Natl Acad Sci USA 1983;80:273–7.
19. Lynch DM, Kay PH. Studies on the polymorphism of the fifth component of complement in laboratory mice. Exp Clin Immunogenet 1995;12:253–60.
20. Kikutani H, Makino S. The murine autoimmune diabetes model: NOD and related strains. Adv Immunol 1992;51:285–322.
21. Yoshida K, Kikutani H. Genetic and immunological basis of autoimmune diabetes in the NOD mouse. Rev Immunogenet 2000;2:140–6.
22. Adorini L, Gregori S, Harrison LC. Understanding autoimmune diabetes: insights from mouse models. Trends Mol Med 2002;8:31–8.
23. Hayward AR, Shreiber M. Neonatal injection of CD3 antibody into nonobese diabetic mice reduces the incidence of insulitis and diabetes. J Immunol 1989;143:1555–9.
24. Chatenoud L, Primo J, Bach JF. CD3 antibody-induced dominant self tolerance in overtly diabetic NOD mice. J Immunol 1997;158:2947–54.
25. Varey AM, Hutchings P, O'Reilly L, Hussell T, Waldmann H, Simpson E, et al. The development of insulin-dependent diabetes mellitus in non-obese diabetic mice: the role of CD4+ and CD8+ T cells. Biochem Soc Trans 1991;19:187–91.
26. Sempe P, Bedossa P, Richard MF, Villa MC, Bach JF, Boitard C. Anti-alpha/beta T cell receptor monoclonal antibody provides an efficient therapy for autoimmune diabetes in nonobese diabetic (NOD) mice. Eur J Immunol 1991;21: 1163–9.
27. Lenschow DJ, Ho SC, Sattar H, Rhee L, Gray G, Nabavi N, et al. Differential effects of anti-B7-1 and anti-B7-2 monoclonal antibody treatment on the development of diabetes in the nonobese diabetic mouse. J Exp Med 1995;181:1145–55.
28. Abu-Hadid MM, Lazarovits AI, Madrenas J. Prevention of diabetes mellitus in the non-obese diabetic mouse strain with monoclonal antibodies against the CD45RB molecule. Autoimmunity 2000;32:73–6.
29. Balasa B, Krahl T, Patstone G, Lee J, Tisch R, McDevitt HO, et al. CD40 ligand-CD40 interactions are necessary for the initiation of insulitis and diabetes in nonobese diabetic mice. J Immunol 1997;159:4620–7.
30. Baron JL, Reich EP, Visintin I, Janeway CA, Jr. The pathogenesis of adoptive murine autoimmune diabetes requires an interaction between alpha 4-integrins and vascular cell adhesion molecule-1. J Clin Invest 1994;93:1700–8.
31. Yang XD, Michie SA, Tisch R, Karin N, Steinman L, McDevitt HO. A predominant role of integrin alpha 4 in the spontaneous development of autoimmune diabetes in non-obese diabetic mice. Proc Natl Acad Sci USA 1994;91: 12604–8.
32. Hanninen A, Jaakkola I, Jalkanen S. Mucosal addressin is required for the development of diabetes in nonobese diabetic mice. J Immunol 1998;160:6018–25.
33. Moriyama H, Yokono K, Amano K, Nagata M, Hasegawa Y, Okamoto N, et al. Induction of tolerance in murine autoimmune diabetes by transient blockade of leukocyte function-associated antigen-1/intercellular adhesion molecule-1 pathway. J Immunol 1996;157:3737–43.
34. Calcinaro F, Gambelunghe G, Lafferty KJ. Protection from autoimmune diabetes by adjuvant therapy in the non-obese diabetic mouse: the role of interleukin-4 and interleukin-10. Immunol Cell Biol 1997;75:467–71.
35. Overbergh L, Decallonne B, Waer M, Rutgeerts O, Valckx D, Casteels KM, et al. 1alpha,25-dihydroxyvitamin D3 induces an autoantigen-specific T-helper 1/T-helper 2 immune shift in NOD mice immunized with GAD65 (p524-543). Diabetes 2000;49:1301–7.
36. Sharif S, Arreaza GA, Zucker P, Mi QS, Sondhi J, Naidenko OV, et al. Activation of natural killer T cells by alpha-galactosylceramide treatment prevents the onset and recurrence of autoimmune Type 1 diabetes. Nat Med 2001;7:1057–62.
37. Tominaga Y, Nagata M, Yasuda H, Okamoto N, Arisawa K, Moriyama H, et al. Administration of IL-4 prevents autoimmune diabetes but enhances pancreatic insulitis in NOD mice. Clin Immunol Immunopathol 1998;86:209–18.
38. Pennline KJ, Roque-Gaffney E, Monahan M. Recombinant

 ISSN 0785-3890

human IL-10 prevents the onset of diabetes in the nonobese diabetic mouse. Clin Immunol Immunopathol 1994;71:169–75.
39. Zheng XX, Steele AW, Hancock WW, Stevens AC, Nickerson PW, Roy-Chaudhury P, et al. A noncytolytic IL-10/Fc fusion protein prevents diabetes, blocks autoimmunity, and promotes suppressor phenomena in NOD mice. J Immunol 1997;158:4507–13.
40. Reddy S, Bibby NJ, Elliott RB. Early nicotinamide treatment in the NOD mouse: effects on diabetes and insulitis suppression and autoantibody levels. Diabetes Res 1990;15: 95–102.
41. Atkinson MA, Maclaren NK, Luchetta R. Insulitis and diabetes in NOD mice reduced by prophylactic insulin therapy. Diabetes 1990;39:933–7.
42. Muir A, Peck A, Clare-Salzler M, Song Y-H, Cornelius J, Luchetta R, et al. Insulin immunization of nonobese diabetic mice induces a protective insulitis characterized by diminished intraislet interferon-γ transcription. J Clin Invest 1995; 95:628–34.
43. Zhang ZJ, Davidson L, Eisenbarth G, Weiner HL. Suppression of diabetes in nonobese diabetic mice by oral administration of porcine insulin. Proc Natl Acad Sci USA 1991;88: 10252–6.
44. Bergerot I, Fabien N, Mayer A, Thivolet C. Active suppression of diabetes after oral administration of insulin is determined by antigen dosage. Ann N Y Acad Sci 1996;778: 362–7.
45. Harrison LC, Dempsey-Collier M, Kramer DR, Takahashi K. Aerosol insulin induces regulatory CD8 gamma delta T cells that prevent murine insulin-dependent diabetes. J Exp Med 1996;184:2167–74.
46. Daniel D, Wegmann DR. Protection of nonobese diabetic mice from diabetes by intranasal or subcutaneous administration of insulin peptide B-(9–23). Proc Natl Acad Sci USA 1996;93:956–60.
47. Urbanek-Ruiz I, Ruiz PJ, Paragas V, Garren H, Steinman L, Fathman CG. Immunization with DNA encoding an immunodominant peptide of insulin prevents diabetes in NOD mice. Clin Immunol 2001;100:164–71.
48. Tian J, Atkinson MA, Clare-Salzler M, Herschenfeld A, Forsthuber T, Lehmann PV. Nasal administration of glutamate decarboxylase (GAD65) peptides induces Th2 responses and prevents murine insulin-dependent diabetes. J Exp Med 1996;183:1561–7.
49. Balasa B, Boehm BO, Fortnagel A, Karges W, Van Gunst K, Jung N, et al. Vaccination with glutamic acid decarboxylase plasmid DNA protects mice from spontaneous autoimmune diabetes and B7/CD28 costimulation circumvents that protection. Clin Immunol 2001;99:241–52.
50. Tisch R, Wang B, Weaver DJ, Liu B, Bui T, Arthos J, et al. Antigen-specific mediated suppression of beta cell autoimmunity by plasmid DNA vaccination. J Immunol 2001; 166:2122–32.
51. Tisch R, Yang XD, Singer SM, Liblau RS, Fugger L, McDevitt HO. Immune response to glutamic acid decarboxylase correlates with insulitis in non-obese diabetic mice. Nature 1993;366:72–5.
52. Elias D, Cohen IR. Peptide therapy for diabetes in NOD mice. Lancet 1994;343:704–6.
53. Blanas E, Carbone FR, Allison J, Miller JF, Heath WR. Induction of autoimmune diabetes by oral administration of autoantigen. Science 1996;274:1707–9.
54. Hanninen A, Braakhuis A, Heath WR, Harrison LC. Mucosal antigen primes diabetogenic cytotoxic T-lymphocytes regardless of dose or delivery route. Diabetes 2001;50: 771–5.
55. Hanninen A, Martinez NR, Davey GM, Heath WR, Harrison LC. Transient blockade of CD40 ligand dissociates pathogenic from protective mucosal immunity. J Clin Invest 2002;109:261–7.
56. von Herrath MG, Whitton JL. DNA vaccination to treat autoimmune diabetes. Ann Med 2000;32:285–92.
57. Woodle ES, Bluestone JA, Zivin RA, Jolliffe LK, Auger J, Xu D, et al. Humanized, nonmitogenic OKT3 antibody, huOKT3 gamma(Ala-Ala): initial clinical experience. Transplant Proc 1998;30:1369–70.
58. Herold KC, Bluestone JA, Montag AG, Parihar A, Wiegner A, Gress RE, et al. Prevention of autoimmune diabetes with nonactivating anti-CD3 monoclonal antibody. Diabetes 1992;41:385–91.
59. Chatenoud L, Thervet E, Primo J, Bach JF. Anti-CD3 antibody induces long-term remission of overt autoimmunity in nonobese diabetic mice. Proc Natl Acad Sci USA 1994;91: 123–7.
60. Smith JA, Tso JY, Clark MR, Cole MS, Bluestone JA. Nonmitogenic anti-CD3 monoclonal antibodies deliver a partial T cell receptor signal and induce clonal anergy. J Exp Med 1997;185:1413–22.
61. Smith JA, Tang Q, Bluestone JA. Partial TCR signals delivered by FcR-nonbinding anti-CD3 monoclonal antibodies differentially regulate individual Th subsets. J Immunol 1998;160:4841–9.
62. Elias D, Meilin A, Ablamunits V, Birk OS, Carmi P, Konen-Waisman S, et al. Hsp60 peptide therapy of NOD mouse diabetes induces a Th2 cytokine burst and downregulates autoimmunity to various beta-cell antigens. Diabetes 1997; 46:758–64.
63. Raz I, Elias D, Avron A, Tamir M, Metzger M, Cohen IR. Beta-cell function in new-onset type 1 diabetes and immunomodulation with a heat-shock protein peptide (DiaPep277): a randomised, double-blind, phase II trial. Lancet 2001;358: 1749–53.
64. Gotfredsen CF, Buschard K, Frandsen EK. Reduction of diabetes incidence of BB Wistar rats by early prophylactic insulin treatment of diabetes-prone animals. Diabetologia 1985;28:933–5.
65. Keller RJ, Eisenbarth GS, Jackson RA. Insulin prophylaxis in individuals at high risk of type I diabetes. Lancet 1993;341: 927–8.
66. Miller A, Lider O, Weiner HL. Antigen-driven bystander suppression after oral administration of antigens. J Exp Med 1991;174:791–8.
67. Gallichan WS, Rosenthal KL. Long-lived cytotoxic T lymphocyte memory in mucosal tissues after mucosal but not systemic immunization. J Exp Med 1996;184:1879–90.
68. Heller B, Wang ZQ, Wagner EF, Radons J, Burkle A, Fehsel K, et al. Inactivation of the poly(ADP-ribose) polymerase gene affects oxygen radical and nitric oxide toxicity in islet cells. J Biol Chem 1995;270:11176–80.
69. Pozzilli P, Visalli N, Signore A, Baroni MG, Buzzetti R, Cavallo MG, et al. Double blind trial of nicotinamide in recent-onset IDDM (the IMDIAB III study). Diabetologia 1995;38:848–52.
70. Chase HP, Butler-Simon N, Garg S, McDuffie M, Hoops SL, O'Brien D. A trial of nicotinamide in newly diagnosed patients with type 1 (insulin-dependent) diabetes mellitus. Diabetologia 1990;33:444–6.
71. Mendola G, Casamitjana R, Gomis R. Effect of nicotinamide therapy upon B-cell function in newly diagnosed type 1 (insulin-dependent) diabetic patients. Diabetologia 1989;32: 160–2.
72. Lampeter EF, Klinghammer A, Scherbaum WA, Heinze E, Haastert B, Giani G, et al. The Deutsche Nicotinamide Intervention Study: an attempt to prevent type 1 diabetes. DENIS Group. Diabetes 1998;47:980–4.

 ISSN 0785-3890

73. Elliott RB, Pilcher CC, Fergusson DM, Stewart AW. A population based strategy to prevent insulin-dependent diabetes using nicotinamide. J Pediatr Endocrinol Metab 1996;9:501–9.
74. Brod SA, Malone M, Darcan S, Papolla M, Nelson L. Ingested interferon alpha suppresses type I diabetes in non-obese diabetic mice. Diabetologia 1998;41:1227–32.
75. Brod SA, Atkinson M, Lavis VR, Brosnan PG, Hardin DS, Orlander PR, et al. Ingested IFN-alpha preserves residual beta cell function in type 1 diabetes. J Interferon Cytokine Res 2001;21:1021–30.
76. Fleischmann WR, Jr, Koren S, Fleischmann CM. Orally administered interferons exert their white blood cell suppressive effects via a novel mechanism. Proc Soc Exp Biol Med 1992;201:200–7.
77. Stewart TA, Hultgren B, Huang X, Pitts-Meek S, Hully J, MacLachlan NJ. Induction of type I diabetes by interferon-alpha in transgenic mice. Science 1993;260:1942–6.
78. Cunningham-Rundles S, Ahrne S, Bengmark S, Johann-Liang R, Marshall F, Metakis L, et al. Probiotics and immune response. Am J Gastroenterol 2000;95:S22–5.
79. Kukreja A, Cost G, Marker J, Zhang C, Sun Z, Lin-Su K, et al. Multiple immuno-regulatory defects in type-1 diabetes. J Clin Invest 2002;109:131–40.
80. Wilson SB, Kent SC, Patton KT, Orban T, Jackson RA, Exley M, et al. Extreme Th1 bias of invariant Valpha24JalphaQ T cells in type 1 diabetes. Nature 1998; 391:177–81.
81. Lee PT, Putnam A, Benlagha K, Teyton L, Gottlieb PA, Bendelac A. Testing the NKT cell hypothesis of human IDDM pathogenesis. J Clin Invest 2002;110:793–800.
82. Hammond KJ, Poulton LD, Palmisano LJ, Silveira PA, Godfrey DI, Baxter AG. alpha/beta-T cell receptor (TCR)+CD4-CD8- (NKT) thymocytes prevent insulin-dependent diabetes mellitus in nonobese diabetic (NOD)/Lt mice by the influence of interleukin (IL)-4 and/or IL-10. J Exp Med 1998;187:1047–56.
83. Lehuen A, Lantz O, Beaudoin L, Laloux V, Carnaud C, Bendelac A, et al. Overexpression of natural killer T cells protects Valpha14- Jalpha281 transgenic nonobese diabetic mice against diabetes. J Exp Med 1998;188:1831–9.
84. Matsuzaki T, Nagata Y, Kado S, Uchida K, Kato I, Hashimoto S, et al. Prevention of onset in an insulin-dependent diabetes mellitus model, NOD mice, by oral feeding of Lactobacillus casei. APMIS 1997;105:643–9.
85. Hessle C, Hanson LA, Wold AE. Lactobacilli from human gastrointestinal mucosa are strong stimulators of IL-12 production. Clin Exp Immunol 1999;116:276–82.
86. Haskins K, Wegmann D. Diabetogenic T-cell clones. Diabetes 1996;45:1299–305.
87. Wegmann DR, Norbury-Glaser M, Daniel D. Insulin-specific T cells are a predominant component of islet infiltrates in pre-diabetic NOD mice. Eur J Immunol 1994;24:1853–7.
88. Daniel D, Gill RG, Schloot N, Wegmann D. Epitope specificity, cytokine production profile and diabetogenic activity of insulin-specific T cell clones isolated from NOD mice. Eur J Immunol 1995;25:1056–62.
89. Alleva DG, Gaur A, Jin L, Wegmann D, Gottlieb PA, Pahuja A, et al. Immunological characterization and therapeutic activity of an altered-peptide ligand, NBI-6024, based on the immunodominant type 1 diabetes autoantigen insulin B-chain (9–23) peptide. Diabetes 2002;51:2126–34.
90. Nicholson LB, Kuchroo VK. T cell recognition of self and altered self antigens. Crit Rev Immunol 1997;17:449–62.
91. Young DA, Lowe LD, Booth SS, Whitters MJ, Nicholson L, Kuchroo VK, et al. IL-4, IL-10, IL-13, and TGF-beta from an altered peptide ligand-specific Th2 cell clone down-regulate adoptive transfer of experimental autoimmune encephalomyelitis. J Immunol 2000;164:3563–72.
92. Veldman CM, Cantorna MT, DeLuca HF. Expression of 1,25-dihydroxyvitamin D(3) receptor in the immune system. Arch Biochem Biophys 2000;374:334–8.
93. Lemire JM, Archer DC, Beck L, Spiegelberg HL. Immunosuppressive actions of 1,25-dihydroxyvitamin D3: preferential inhibition of Th1 functions. J Nutr 1995;125:1704S–8S.
94. Penna G, Adorini L. 1 Alpha,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. J Immunol 2000;164:2405–11.
95. Griffin MD, Lutz W, Phan VA, Bachman LA, McKean DJ, Kumar R. Dendritic cell modulation by 1alpha,25 dihydroxyvitamin D3 and its analogs: a vitamin D receptor-dependent pathway that promotes a persistent state of immaturity in vitro and in vivo. Proc Natl Acad Sci USA 2001;98:6800–5.
96. Mathieu C, Waer M, Casteels K, Laureys J, Bouillon R. Prevention of type I diabetes in NOD mice by nonhypercalcemic doses of a new structural analog of 1,25-dihydroxyvitamin D3, KH1060. Endocrinology 1995;136:866–72.
97. Adorini L, Penna G, Giarratana N, Uskokovic M. Tolerogenic dendritic cells induced by vitamin D receptor ligands enhance regulatory T cells inhibiting allograft rejection and autoimmune diseases. J Cell Biochem 2003;88:227–33.
98. Karges W, Hammond-McKibben D, Cheung RK, Visconti M, Shibuya N, Kemp D, et al. Immunological aspects of nutritional diabetes prevention in NOD mice: a pilot study for the cow's milk-based IDDM prevention trial. Diabetes 1997;46:557–64.
99. Bodington MJ, McNally PG, Burden AC. Cow's milk and type 1 childhood diabetes: no increase in risk. Diabet Med 1994;11:663–5.
100. Fava D, Leslie RD, Pozzilli P. Relationship between dairy product consumption and incidence of IDDM in childhood in Italy. Diabetes Care 1994;17:1488–90.
101. Mathis D, Vence L, Benoist C. beta-Cell death during progression to diabetes. Nature 2001;414:792–8.
102. Miller JFAP, Flavell RA. T cell tolerance and autoimmunity in transgenic models of central and peripheral tolerance. Curr Opin Immunol 1994;6:892–9.
103. Kurts C. Cross-presentation: inducing CD8 T cell immunity and tolerance. J Mol Med 2000;78:326–32.
104. Mintern J, Li M, Davey GM, Blanas E, Kurts C, Carbone FR, et al. The use of carboxyfluorescein diacetate succinimidyl ester to determine the site, duration and cell type responsible for antigen presentation in vivo. Immunol Cell Biol 1999;77:539–43.
105. Blanas E, Davey GM, Carbone FR, Heath WR. A bone marrow-derived APC in the gut-associated lymphoid tissue captures oral antigens and presents them to both CD4+ and CD8+ T cells. J Immunol 2000;164:2890–6.
106. Arnold B, Goodnow C, Hengartner H, Hammerling G. The coming of transgenic mice: tolerance and immune reactivity. Immunol Today 1990;11:69–72.
107. Heath WR, Allison J, Hoffmann MW, Schonrich G, Hammerling G, Arnold B, et al. Autoimmune diabetes as a consequence of locally produced interleukin-2. Nature 1992; 359:547–9.
108. Kurts C, Heath WR, Carbone FR, Allison J, Miller JF, Kosaka H. Constitutive class I-restricted exogenous presentation of self antigens in vivo. J Exp Med 1996;184:923–30.
109. Kreuwel HT, Aung S, Silao C, Sherman LA. Memory CD8(+) T cells undergo peripheral tolerance. Immunity 2002;17:73–81.
110. Huang AY, Golumbek P, Ahmadzadeh M, Jaffee E, Pardoll D, Levitsky H. Role of bone marrow-derived cells in

 ISSN 0785-3890

presenting MHC class I-restricted tumor antigens. Science 1994;264:961–5.
111. Sigal LJ, Crotty S, Andino R, Rock KL. Cytotoxic T-cell immunity to virus-infected non-haematopoietic cells requires presentation of exogenous antigen. Nature 1999;398:77–80.
112. Mueller SN, Jones CM, Smith CM, Heath WR, Carbone FR. Rapid cytotoxic T lymphocyte activation occurs in the draining lymph nodes after cutaneous herpes simplex virus infection as a result of early antigen presentation and not the presence of virus. J Exp Med 2002;195:651–6.
113. Liblau RS, Wong FS, Mars LT, Santamaria P. Autoreactive CD8 T cells in organ-specific autoimmunity: emerging targets for therapeutic intervention. Immunity 2002;17:1–6.
114. Wong FS, Karttunen J, Dumont C, Wen L, Visintin I, Pilip IM, et al. Identification of an MHC class I-restricted autoantigen in type 1 diabetes by screening an organ-specific cDNA library. Nat Med 1999;5:1026–31.
115. Verdaguer J, Schmidt D, Amrani A, Anderson B, Averill N, Santamaria P. Spontaneous autoimmune diabetes in monoclonal T cell nonobese diabetic mice. J Exp Med 1997;186: 1663–76.
116. DiLorenzo TP, Graser RT, Ono T, Christianson GJ, Chapman HD, Roopenian DC, et al. Major histocompatibility complex class I-restricted T cells are required for all but the end stages of diabetes development in nonobese diabetic mice and use a prevalent T cell receptor alpha chain gene rearrangement. Proc Natl Acad Sci USA 1998;95:12538–43.
117. Hamilton-Williams EE, Serreze DV, Charlton B, Johnson EA, Marron MP, Mullbacher A, et al. Transgenic rescue implicates beta2-microglobulin as a diabetes susceptibility gene in nonobese diabetic (NOD) mice. Proc Natl Acad Sci USA 2001;98:11533–8.
118. Graser RT, DiLorenzo TP, Wang F, Christianson GJ, Chapman HD, Roopenian DC, et al. Identification of a CD8 T cell that can independently mediate autoimmune diabetes development in the complete absence of CD4 T cell helper functions. J Immunol 2000;164:3913–8.
119. Kanagawa O, Shimizu J, Vaupel BA. Thymic and postthymic regulation of diabetogenic CD8 T cell development in TCR transgenic nonobese diabetic (NOD) mice. J Immunol 2000; 164:5466–73.
120. Hoglund P, Mintern J, Waltzinger C, Heath W, Benoist C, Mathis D. Initiation of autoimmune diabetes by developmentally regulated presentation of islet cell antigens in the pancreatic lymph nodes. J Exp Med 1999;189:331–9.
121. Finegood DT, Scaglia L, Bonner-Weir S. Dynamics of beta-cell mass in the growing rat pancreas. Estimation with a simple mathematical model. Diabetes 1995;44:249–56.
122. Kassem SA, Ariel I, Thornton PS, Scheimberg I, Glaser B. Beta-cell proliferation and apoptosis in the developing normal human pancreas and in hyperinsulinism of infancy. Diabetes 2000;49:1325–33.
123. Trudeau JD, Dutz JP, Arany E, Hill DJ, Fieldus WE, Finegood DT. Neonatal beta-cell apoptosis: a trigger for autoimmune diabetes? Diabetes 2000;49:1–7.
124. Albert ML, Sauter B, Bhardwaj N. Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. Nature 1998;392:86–9.
125. Kurts C, Miller JF, Subramaniam RM, Carbone FR, Heath WR. Major histocompatibility complex class I-restricted cross-presentation is biased towards high dose antigens and those released during cellular destruction. J Exp Med 1998; 188:409–14.
126. Iyoda T, Shimoyama S, Liu K, Omatsu Y, Akiyama Y, Maeda Y, et al. The CD8+ dendritic cell subset selectively endocytoses dying cells in culture and in vivo. J Exp Med 2002;195:1289–302.
127. Steinman RM, Turley S, Mellman I, Inaba K. The induction of tolerance by dendritic cells that have captured apoptotic cells. J Exp Med 2000;191:411–6.
128. Vezys V, Lefrancois L. Cutting edge: inflammatory signals drive organ-specific autoimmunity to normally cross-tolerizing endogenous antigen. J Immunol 2002;169:6677–80.
129. Heath WR, Carbone FR. Cross-presentation in viral immunity and self-tolerance. Nat Rev Immunol 2001;1:126–34.
130. Ochsenbein AF, Klenerman P, Karrer U, Ludewig B, Pericin M, Hengartner H, et al. Immune surveillance against a solid tumor fails because of immunological ignorance. Proc Natl Acad Sci USA 1999;96:2233–8.
131. Morgan DJ, Kreuwel HT, Sherman LA. Antigen concentration and precursor frequency determine the rate of CD8+ T cell tolerance to peripherally expressed antigens. J Immunol 1999;163:723–7.
132. Kurts C, Sutherland RM, Davey G, Li M, Lew AM, Blanas E, et al. CD8 T cell ignorance or tolerance to islet antigens depends on antigen dose. Proc Natl Acad Sci USA 1999;96: 12703–7.
133. Ohashi PS. T-cell signalling and autoimmunity: molecular mechanisms of disease. Nat Rev Immunol 2002;2:427–38.
134. Ohashi PS, Oehen S, Buerki K, Pircher H, Ohashi CT, Odermatt B, et al. Ablation of 'tolerance' and induction of diabetes by virus infection in viral antigen transgenic mice. Cell 1991;65:305–17.
135. Oldstone MB, Nerenberg M, Southern P, Price J, Lewicki H. Virus infection triggers insulin-dependent diabetes mellitus in a transgenic model: role of anti-self (virus) immune response. Cell 1991;65:319–31.
136. Benoist C, Mathis D. Autoimmunity provoked by infection: how good is the case for T cell epitope mimicry? Nat Immunol 2001;2:797–801.
137. Schwartz RH. T Cell Anergy. Annu Rev Immunol 2003;21: 305–34.
138. Schonrich G, Kalinke U, Momburg F, Malissen M, Schmitt-Verhulst AM, Malissen B, et al. Down-regulation of T cell receptors on self-reactive T cells as a novel mechanism for extrathymic tolerance induction. Cell 1991;65:293–304.
139. Kurts C, Carbone FR, Barnden M, Blanas E, Allison J, Heath WR, et al. CD4+ T cell help impairs CD8+ T cell deletion induced by cross-presentation of self-antigens and favors autoimmunity. J Exp Med 1997;186:2057–62.
140. Adler AJ, Marsh DW, Yochum GS, Guzzo JL, Nigam A, Nelson WG, et al. CD4+ T cell tolerance to parenchymal self-antigens requires presentation by bone marrow-derived antigen-presenting cells. J Exp Med 1998;187:1555–64.
141. Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. Nature 1996;383:787–93.
142. Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol 1989;7:145–73.
143. Sakaguchi S, Sakaguchi N, Shimizu J, Yamazaki S, Sakihama T, Itoh M, et al. Immunologic tolerance maintained by CD25+ CD4+ regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. Immunol Rev 2001;182:18–32.
144. Grewal IS, Grewal KD, Wong FS, Wang H, Picarella DE, Janeway CA, Jr., et al. Expression of transgene encoded TGF-beta in islets prevents autoimmune diabetes in NOD mice by a local mechanism. J Autoimmun 2002;19:9–22.
145. Salomon B, Bluestone JA. Complexities of CD28/B7: CTLA-4 costimulatory pathways in autoimmunity and transplantation. Annu Rev Immunol 2001;19:225–52.
146. Green EA, Eynon EE, Flavell RA. Local expression of TNFalpha in neonatal NOD mice promotes diabetes by enhancing presentation of islet antigens. Immunity 1998;9: 733–43.

 ISSN 0785-3890

147. Amrani A, Serra P, Yamanouchi J, Han B, Thiessen S, Verdaguer J, et al. CD154-dependent priming of diabetogenic CD4(+) T cells dissociated from activation of antigen-presenting cells. Immunity 2002;16:719–32.
148. Wong FS, Visintin I, Wen L, Granata J, Flavell R, Janeway CA. The role of lymphocyte subsets in accelerated diabetes in nonobese diabetic-rat insulin promoter-B7-1 (NOD-RIP-B7-1) mice. J Exp Med 1998;187:1985–93.
149. Yang XD, Tisch R, Singer SM, Cao ZA, Liblau RS, Schreiber RD, et al. Effect of tumor necrosis factor alpha on insulin-dependent diabetes mellitus in NOD mice. I. The early development of autoimmunity and the diabetogenic process. J Exp Med 1994;180:995–1004.
150. Green EA, Flavell RA. The temporal importance of TNFalpha expression in the development of diabetes. Immunity 2000;12:459–69.
151. Green EA, Choi Y, Flavell RA. Pancreatic lymph node-derived CD4(+)CD25(+) Treg cells: highly potent regulators of diabetes that require TRANCE-RANK signals. Immunity 2002;16:183–91.
152. Salomon B, Lenschow DJ, Rhee L, Ashourian N, Singh B, Sharpe A, et al. B7/CD28 costimulation is essential for the homeostasis of the CD4+CD25+ immunoregulatory T cells that control autoimmune diabetes. Immunity 2000;12:431–40.
153. Thompson CB, Allison JP. The emerging role of CTLA-4 as an immune attenuator. Immunity 1997;7:445–50.
154. Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, et al. A $CD4^+$ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Nature 1997; 389:737–42.
155. Letterio JJ, Roberts AB. Regulation of immune responses by TGF-beta. Annu Rev Immunol 1998;16:137–61.
156. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 2001;19:683–765.
157. Medzhitov R, Janeway CA, Jr. Decoding the patterns of self and nonself by the innate immune system. Science 2002;296: 298–300.
158. Garside P, Mowat AM. Mechanisms of oral tolerance. Crit Rev Immunol 1997;17:119–37.
159. Pape KA, Merica R, Mondino A, Khoruts A, Jenkins MK. Direct evidence that functionally impaired CD4+ T cells persist in vivo following induction of peripheral tolerance. J Immunol 1998;160:4719–29.
160. Appel H, Seth NP, Gauthier L, Wucherpfennig KW. Anergy induction by dimeric TCR ligands. J Immunol 2001;166:5279–85.
161. Casares S, Hurtado A, McEvoy RC, Sarukhan A, von Boehmer H, Brumeanu TD. Down-regulation of diabetogenic CD4+ T cells by a soluble dimeric peptide-MHC class II chimera. Nat Immunol 2002;3:383–91.
162. Hackstein H, Morelli AE, Thomson AW. Designer dendritic cells for tolerance induction:guided not misguided missiles. Trends Immunol 2001;22:437–42.
163. Ashkenazi A, Dixit VM. Apoptosis control by death and decoy receptors. Curr Opin Cell Biol 1999;11:255–60.
164. Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med 2000;192:1027–34.
165. Latchman Y, Wood CR, Chernova T, Chaudhary D, Borde M, Chernova I, et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. Nat Immunol 2001;2:261–8.
166. Min WP, Gorczynski R, Huang XY, Kushida M, Kim P, Obataki M, et al. Dendritic cells genetically engineered to express Fas ligand induce donor-specific hyporesponsiveness and prolong allograft survival. J Immunol 2000;164:161–7.
167. Matsue H, Matsue K, Walters M, Okumura K, Yagita H, Takashima A. Induction of antigen-specific immunosuppression by CD95L cDNA-transfected 'killer' dendritic cells. Nat Med 1999;5:930–7.
168. Buonocore S, Van Meirvenne S, Demoor FX, Paulart F, Thielemans K, Goldman M, et al. Dendritic cells transduced with viral interleukin 10 or Fas ligand: no evidence for induction of allotolerance in vivo. Transplantation 2002;73: S27–30.
169. Maksimow M, Santanen M, Jalkanen S, Hanninen A. Responding naive T cells differ in their sensitivity to Fas-engagement: early death of many T cells is compensated by costimulation of surviving T cells. Blood 2003:in press.
170. Mellman I, Steinman RM. Dendritic cells: specialized and regulated antigen processing machines. Cell 2001;106:255–8.
171. Falcone M, Lee J, Patstone G, Yeung B, Sarvetnick N. B lymphocytes are crucial antigen-presenting cells in the pathogenic autoimmune response to GAD65 antigen in nonobese diabetic mice. J Immunol 1998;161:1163–8.
172. King C, Davies J, Mueller R, Lee MS, Krahl T, Yeung B, et al. TGF-beta1 alters APC preference, polarizing islet antigen responses toward a Th2 phenotype. Immunity 1998;8:601–13.
173. Caux C, Massacrier C, Vanbervliet B, Dubois B, de Saint-Vis B, Dezutter-Dambuyant C, et al. CD34+ hematopoietic progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to GM-CSF+TNF alpha. Adv Exp Med Biol 1997;417:21–5.
174. Markowicz S, Engleman EG. Granulocyte-macrophage colony-stimulating factor promotes differentiation and survival of human peripheral blood dendritic cells in vitro. J Clin Invest 1990;85:955–61.
175. Dhodapkar MV, Steinman RM, Sapp M, Desai H, Fossella C, Krasovsky J, et al. Rapid generation of broad T-cell immunity in humans after a single injection of mature dendritic cells. J Clin Invest 1999;104:173–80.
176. Dhodapkar MV, Steinman RM, Krasovsky J, Munz C, Bhardwaj N. Antigen-specific inhibition of effector T cell function in humans after injection of immature dendritic cells. J Exp Med 2001;193:233–8.
177. Donnelly JJ, Ulmer JB, Shiver JW, Liu MA. DNA vaccines. Annu Rev Immunol 1997;15:617–48.
178. Ulmer JB, Donnelly JJ, Parker SE, Rhodes GH, Felgner PL, Dwarki VJ, et al. Heterologous protection against influenza by injection of DNA encoding a viral protein. Science 1993; 259:1745–9.
179. Karges W, Pechhold K, Al Dahouk S, Riegger I, Rief M, Wissmann A, et al. Induction of autoimmune diabetes through insulin (but not GAD65) DNA vaccination in nonobese diabetic and in RIP-B7.1 mice. Diabetes 2002;51: 3237–44.
180. Quintana FJ, Carmi P, Cohen IR. DNA vaccination with heat shock protein 60 inhibits cyclophosphamide-accelerated diabetes. J Immunol 2002;169:6030–5.
181. Quintana FJ, Rotem A, Carmi P, Cohen IR. Vaccination with empty plasmid DNA or CpG oligonucleotide inhibits diabetes in nonobese diabetic mice: modulation of spontaneous 60-kDa heat shock protein autoimmunity. J Immunol 2000;165:6148–55.
182. Tighe H, Corr M, Roman M, Raz E. Gene vaccination: plasmid DNA is more than just a blueprint. Immunol Today 1998;19:89–97.
183. Schirmbeck R, Reimann J. Revealing the potential of DNA-based vaccination: lessons learned from the hepatitis B virus surface antigen. Biol Chem 2001;382:543–52.
184. Stevenson FK, Rosenberg W. DNA vaccination: a potential weapon against infection and cancer. Vox Sang 2001;80:12–8.
185. Bonifaz L, Bonnyay D, Mahnke K, Rivera M, Nussenzweig MC, Steinman RM. Efficient targeting of protein antigen to

 ISSN 0785-3890

the dendritic cell receptor DEC-205 in the steady state leads to antigen presentation on major histocompatibility complex class I products and peripheral CD8+ T cell tolerance. J Exp Med 2002;196:1627–38.

186. Burt RK, Slavin S, Burns WH, Marmont AM. Induction of tolerance in autoimmune diseases by hematopoietic stem cell transplantation: getting closer to a cure? Blood 2002;99:768–84.

 ISSN 0785-3890